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VERTEBRATE EMBRYOLOGY

VERTEBRATE EMBRYOLOGY

A TEXTBOOK FOR COLLEGES AND UNIVERSITIES

BY

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SECOND EDITION, THOROUGHLY REVISED

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PREFACE TO SECOND EDITION

The favorable reception accorded the first edition of this book has encouraged the author to prepare this revision, incorporating such changes as have been suggested by his own experience with it as well as those that have been called to his attention by other teachers. In addition to the correction of errata, the reader will note that the scientific terminology has been considerably reduced. Not only have the synonyms been excluded as before but in this revision many of the more technical terms also have been discarded.

A few new figures have been introduced, several have been redrawn, and all the drawings in the atlas of sections (Part III) have been reproduced by a method which more nearly approximates the artist's originals.

To all those who have been kind enough to advise the author of errors in the original edition, he is deeply grateful, but realizing the impossibility that all such blunders have been brought to light, he will welcome correspondence from others who have suggestions to offer.

W. S.

PREFACE TO FIRST EDITION

This book is intended to serve as an introduction to the study of Vertebrate Embryology for undergraduate students in colleges and universities. For this study they have been prepared by completing introductory courses in the principles of biology and the anatomy of the vertebrates. It has been the writer's aim to correlate embryological principles as discussed in lecture and classroom with the anatomy of vertebrate embryos as studied in the laboratory, in such a manner as to produce a text which should be both practical and teachable.

After a general introduction to the subject, a large part of the text is devoted to the subject of early embryology, making use of the comparative method which has been found most successful in the experience of many teachers. Especial emphasis has been laid on four forms: Amphioxus, because of the beautiful and diagrammatic simplicity with which the early stages may be seriated; the frog, long an object of laboratory study; the chick, always available for laboratory preparation and observation; and man, whenever human material is available. Following this section, which includes the period of germ-layer formation, the embryonic membranes, and the development of body form, a second division of the bock deals with the derivation of the separate organs and organ systems from the germ layers. Here, too, the method is essentially comparative. The general plan by which each organ system develops is first sketched in broad outlines, followed by an account of the divergent details in the frog, chick, and man.

The remainder of the book is intended for laboratory use. In the third section is given a succinct account of the anatomy of each of the more commonly studied stages in the development of the frog, chick, and pig, illustrated by figures of whole mounts and sections selected from the splendid collections at the University of Illinois built up by Professor J. S. Kingsley. The writer has followed the sequential or chronological method in this

section as it has been his experience that this method is as successful in the laboratory as is the comparative method in the classroom. It is hoped that from the study of the transparent whole mounts, as well as the transverse, sagittal, and frontal sections, the student may be enabled to visualize the anatomy of embryos in three dimensions. The concluding section of the text deals with methods of preparing embryos for study and of instructions in elementary methods of embryological study. In the writer's experience it is much easier for the student to grasp the relationship of serial sections after he has prepared a set of his own.

In view of the fact that this book has been written primarily for the undergraduate, the writer feels that he need offer no apology for the omission of historical reviews, controversial discussions of obscure phenomena, references to original sources, or lists of synonyms. These neither interest nor profit the beginning student. In the concluding division of the introduction may be found a carefully selected list of handbooks, texts, and atlases to which the more ambitious student may be referred, while a list of references for collateral reading follows each chapter. The brevity of the text is intentional. If the student is informed that every word and sentence is an integral part of the story, he will master it in detail rather than attempt to pick out the more salient points, a procedure for which he is hardly prepared as yet. Brief summaries are appended to the chapters in Parts I and II.

To compensate for the brevity of the text, the reader is provided with a profusion of illustrations, prepared by the well-known scientific artist, Mrs. Katharine Hill Paul. To her skill the writer is deeply indebted. Attention may be called to the fact that no abbreviations are employed in the labeling of figures. The beginning student will be apt to study the illustrations more carefully if he is not compelled to search through lengthy legends to interpret them. Professor E. B. Wilson and Professor J. S. Kingsley have kindly allowed the writer to have several figures from their writings redrawn in order that these might conform to the general style of this text. Messrs. W. B. Saunders have generously consented to the reproduction of certain figures, from " Developmental Anatomy " by Professor L. B. Arey, which

had been drawn by Mrs. Paul for that text. The writer is indebted also to Professor S. H. Gage and the Comstock Publishing Company for the use of an electrotype from " The Microscope." The source of all illustrations not original in this text is acknowledged in the legends.

It is a pleasure to record here a debt of gratitude to Professor J. H. McGregor and Professor J. S. Kingsley for their kindness in reading the original manuscript. Professor H. B. Ward has generously placed the resources of the Department of Zoölogy at the writer's disposal during the preparation of this book. Dr. A. R. Cahn has contributed preparations, suggestions, and — most appreciated of all — uncounted hours in the drudgery of proof-reading and indexing. For assistance in these labors the writer is indebted also to Dr. H. W. Hann and Dr. F. B. Adamstone. If this book serves to help the undergraduate through his first course in embryology, it is due, in no small measure, to the many students who have labored through these pages in mimeographed form and pointed out the difficulties they encountered.

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Urbana.

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VERTEBRATE EMBRYOLOGY

CHAPTER I

INTRODUCTION

Before commencing the study of vertebrate embryology, it is necessary to inquire into the meaning of the words " vertebrate " and " embryology " as well as to set forth the general plan of this text.

A. EMBRYOLOGY

Embryology may be defined as that division of biological science which deals with the development of the individual. By development is meant the complicated series of changes in form and function through which the initial germ becomes transformed into the sexually mature adult. The germ with which development commences is, in the case of the vertebrates at least, a fertilized egg; the sexually mature adult is an animal that has acquired the ability to produce germ cells. Embryology, therefore, deals with the cycle of the life history from germ cell to germ cell. Embryology is sometimes confused with **ontogeny,** which is the study of the entire life history of the individual and includes the period of **senescence,** during which the reproductive powers wane until terminated by death. Contrasted with ontogeny is the subject **phylogeny,** which deals with the history of the race to which an individual belongs.

Content. — Embryology is sometimes called the science of eggs and embryos. These words have very indefinite meanings. Thus we speak of the hen's " egg," whether it is still in the ovary or completing its twenty-first day of incubation, and qualify the noun by the use of such adjectives as " ovarian," " fertilized," " incubated," or the like. Inside this " egg " may be found an embryo. Strictly speaking, the " egg," or **ovum,** is

the mature female germ cell just prior to the completion of fertilization, after which event the term " embryo " should be used up to the time when the egg yolk has been consumed. Except in the case of mammals, this is often about the time when the embryo is hatched from the protective envelopes in which it has been developing. If the amount of yolk is large, as in the hen's egg, the embryonic period is long enough for the young to have acquired many of the adult characters before it is forced to seek a new food supply. If the amount of yolk is small, however, as in the frog's egg, the young hatches in a form quite unlike the adult, e.g., the tadpole. These intermediate forms, which, though immature and usually incapable of reproduction, obtain their food from extraneous sources, are called **larvæ**. The larval period may be quite prolonged as in the bullfrog, which spends two seasons in the tadpole stage before metamorphosis. In rare instances, larvæ, like the axolotl, a larval form of the salamander, *Ambystoma tigrinum*, acquire the power of reproduction. Among the mammals, except in a few egg-laying species, the embryo develops within the body of the mother where, after the consumption of the yolk and the attainment of some of the adult characteristics, it is called a **fœtus.**

Method. — Like any of the biological sciences, embryology may be concerned primarily with the study of form, **morphology,** or with that of function, **physiology.** There are, therefore, two main divisions of embryology, comparative and experimental; the first is primarily morphological, the second physiological.

Comparative embryology. — This division of embryology deals with the changes in form through which the individual passes in development and with a comparison of these form changes in different races of animals. In this connection it must be remembered that the essence of development is continuity and that these changes in form merge imperceptibly from one into another. The stages of development of which we shall speak are obtained by halting the process of development at a given time, much as we might check the projection of a cinema reel in order to obtain a still picture of a particular scene. Certain of these stages are found in the development of all the higher animals and so are known as natural stages and are used to mark divisions in the study of comparative embryology.

Pre-embryonic stages. — The first stage of embryology is the study of the germ cells, or **gametes,** produced by the parent individuals, and the method of the development of these gametes, or **gametogenesis.** This division of embryology actually carries us back into the embryology of the parent animals during the time when the germ cells were developing. The second period recognized by comparative embryology is the very short interval during which two gametes, one male, the other female, unite to form the fertilized egg, or **zygote.** This is known as the period of **fertilization.** As both gametes and zygote are cells, this part of embryology is closely connected with the more general study of cell phenomena, cytology.

Embryonic stages. — After the fertilization of the egg comes the period of embryonic development proper which may be subdivided into three main divisions. The first deals with the period in which the zygote undergoes by an orderly series of divisions into many cells called **blastomeres,** and is known as the period of **cleavage.** Cell division continues throughout the life of the individual, but the period of cleavage is terminated when the blastomeres begin to rearrange themselves in strata called the **germ layers** which may be considered the primary tissues, or aggregates of cells. The time during which these layers are formed is called the germ-layer period. From the germ layers finally arise the various **organs,** or aggregates of tissues by means of which the individual carries on the vital functions. This is called the period of **organogeny.**

The recapitulation theory. — The study of comparative embryology was founded by Von Baer in 1828. He established the fact that, in the series of form changes through which an embryo passes, the more general characters of the race to which the embryo belongs are recognizable before the more special characters appear. In the development of man, for example, vertebrate characters appear before mammalian features; these in turn are visible before the primate characteristics appear; and the distinctive human details are the last to become manifest. Influenced by Darwin's " Origin of Species " (1859), Haeckel interpreted these facts from the standpoint of evolution in the famous recapitulation theory, or, as he called it, the **fundamental biogenetic law:** " Ontogeny is the short and rapid recapitulation

of phylogeny." In other words, the embryo, in passing through the orderly series of form changes which produce first the more general and later the more special characters of the adult, is repeating, in a shortened and altered way, the general evolution of the race from more primitive and simple ancestors to more advanced and complicated descendants.

From this statement it would follow that the evolution of the race might be deduced from a careful investigation of the development of the individual, and spurred by this hope many important embryological researches were undertaken. But certainly in vertebrate embryology, at least, the biogenetic law has not proved generally applicable. The human embryo, though resembling the embryo of a fish or reptile in certain structures at different times, is at no time entirely fish-like or reptilian. Organs such as the pronephros which is functional in the fish and amphibian larva are found in the embryonic stages of higher forms to be sure, but the conservation of these structures is to be explained on the basis of their utility as **organizators** (page 93) or stimuli to the development of other structures rather than as reminiscences of ancient history alone. Even vestigial organs such as the human allantois serve such a purpose, probably in this case in connection with the development of the umbilical (allantoic) blood vessels. It has ever been the history of anatomy to discover that organs once classed as functionless vestiges, like the endocrine glands, have important parts in the economy of the body.

This retention of old structures for new purposes, however, has made it possible from the evidence of comparative embryology to determine the probable course of evolution of the different organs; and the conclusions drawn from this study, when correlated with those obtained from comparative anatomy and palæontology, are all-important in establishing the reality of evolution.

Experimental embryology. — The physiological method of attacking the problems of embryology is to inquire into the mechanism of the developmental processes associated with the series of form changes undergone by the embryo. In experimental embryology, the conditions of development are altered and the end results compared with those following normal development.

Pre-embryonic processes. — It is a remarkable fact that every embryo develops into an adult similar in every essential character to its parents, and yet that no two adults are exactly similar. The sole material connection between offspring and parents is that found in the germ cells, and here embryology comes into close contact with the study of heredity, genetics, for embryology is, in the last analysis, heredity in action. It is one of the triumphs of modern science that in the behavior of the germ cells during gametogenesis and fertilization has been found the explanation of the well-known Mendelian laws which govern the distribution of hereditary characters. In gametogenesis there are distributed to the germ cells minute bodies called **chromosomes** which act as the bearers of hereditary factors, and in fertilization two sets of these are brought together, one from each parent, to be rearranged and dealt out anew to the germ cells formed by the developing individual.

But fertilization is something more than the mere bringing together of two sets of parental chromosomes and potential characters. It initiates development, for without fertilization neither egg nor sperm can normally produce an embryo. In the attempt to analyze this process, embryology is associated with the studies that deal with the physicochemical phenomena of life, biophysics and biochemistry.

Embryonic processes. — To the older embryologists the egg was a simple mass of homogeneous protoplasm which gave rise, step by step, to more complicated structures than those originally present. This theory of the nature of embryogenesis is known as **epigenesis.** The invention of the microscope resulted in the discovery of minute structures in the egg, and some overenthusiastic observers even believed that they could see a miniature adult in the egg or in the sperm. There arose in consequence the theory that all the structures of the adult were contained in miniature in the egg (or sperm) and that development proceeded by the unfolding and growth of these predelineated parts, — the theory of **preformation.** More powerful microscopes and ingenious experiments have led modern embryologists to return to the theory of epigenesis. Five factors are concerned in the epigenetic process:

(1) **Cell division.** Immediately after fertilization the egg divides, commencing again the series of divisions through which it came into existence and by means of which it will give rise to the multicellular organism. During the first few divisions of the embryo, none of the other factors can be demonstrated.

(2) **Growth.** By this we mean an increase in mass, associated with the metabolism of the embryo, for it occurs only when the rate of **anabolism,** the processes by which protoplasm is built up, is greater than that of **katabolism,** the processes by which protoplasm is broken down (oxidized) to release energy. Growth may take place independently of other factors.

(3) **Molding,** a phenomenon in which the embryonic material is reshaped independently of cell division or growth.

(4) **Localization.** This term is applied to the appearance of the different parts of the embryo in a regular order with respect to both time and position. The structure by which the first appearance of any part can be recognized is called its **primordium** (German, *anlage;* French, *ébauche*).

(5) **Differentiation,** or the actual transformation of the primordia into the particular parts of the embryo to which they give rise. Differentiation takes place either by the modification of the structure of the cells, or by that of the tissues, due to the movements of cells, either singly or *en masse.* These movements result in the production of cell aggregates, which may be linear (rod-like), superficial (plate-like), or massive. These aggregates may later be modified by growth, local or general, by atrophy, by subdivision into, or fusion with, other aggregates.

Mechanism and vitalism. — It will have been seen from the foregoing paragraphs that the aim of experimental embryology is to interpret the series of form changes undergone by the embryo in terms of general physiological laws, which in turn are based on the fundamental laws of chemistry and physics. From this point of view the egg is regarded as a " chemical machine " (Loeb), and all its complicated activities are referred to the ordinary process of natural laws. In contrast to this mechanistic interpretation of vital processes should be mentioned the vitalistic conception, which is that beyond and above the ordinary natural laws are supernatural agencies, e.g., the **entelechy** (Driesch), which shape the course of development. The use of

such metaphysical conceptions, however, carries us out of the domain of science and into the realm of philosophy.

B. THE VERTEBRATES

The study of embryology in this book is restricted to that of the vertebrates, those animals that possess a backbone, or rather a series of backbones, known as the vertebral column. Here are included those forms commonly known as the fish, amphibians, reptiles, birds, and mammals. They are a part, by far the largest, of that subdivision of the animal kingdom called the **Phylum Chordata.** This group is distinguished from all others by the possession, at some time during the life history, of an axial supporting rod, the **notochord;** a dorsal central nervous system; and openings in the anterior part of the alimentary canal, the **visceral clefts.** The non-vertebrate members of this phylum are known collectively as **protochordates,** and include the worm-like Balanoglossus, the mollusc-like tunicates (e.g., Cynthia), and the fish-like Amphioxus. We shall have occasion to refer often to the last-named of these protochordates.

The plan of the vertebrate body. — There are certain fundamental characteristics of the vertebrate body which are to be borne in mind while dealing with the embryology of this group.

1. The body is multicellular, composed of innumerable cells, arising from the repeated divisions of the zygote and specialized for widely diverse functions.

2. The parts of the body arise from three germ layers, or aggregates of embryonic cells, which typically form three concentric tubes. The inner tube gives rise to the alimentary canal and its derivatives. The outer tube forms the skin and its derivatives. The middle tube splits into an inner layer, which becomes associated with the alimentary canal, and an outer layer, which becomes associated with the skin, while the space between forms a body cavity known as the **cœlom.**

3. There are three axes of symmetry: (1) from the anterior (cephalic) end to the posterior (caudal) end; (2) from the back (dorsal) surface to the belly (ventral) surface; and (3) from the median axis to either right (dextral) or left (sinistral) side. Right and left sides are mirror images of each other, and the body as a whole is bilaterally symmetrical.

4. Along the main (cephalo-caudal) axis, the body is incompletely divided into a series of similar parts called **metameres.** This metamerism is more marked in structures derived from the middle germ layer.

5. At the anterior end of the body, there is a specialization and condensation of metameres to form the head. This **cephalization** involves especially the entrance into the alimentary canal, the mouth, the higher centers of the nervous system, the brain, and the organs of special sense, the eye, ear, and nose.

6. There are typically two pairs of lateral locomotor appendages; the development of one pair, or of both, may be suppressed, however, as in the case of snakes. Additional dorsal or ventral unpaired appendages may be present, as in fish.

The vertebrate organ systems. — The organs are aggregates of tissues specialized for particular functions in the body. They are grouped in **systems,** each of which has to do with a particular function of the body. A list of the organ systems recognized in the vertebrate body follows:

1. The **integument,** or skin and its derivatives, primarily protective in function.

2. The **sense organs,** which receive from external sources stimuli to which the animal reacts.

3. The **nervous system,** by means of which stimuli from the sense organs are transmitted and the reactions of the body to external stimuli are coördinated.

4. The **muscles,** or contractile elements by means of which the body or its parts may be moved.

5. The **skeleton,** or structural framework of the body.

6. The **digestive system,** including the organs concerned with food.

7. The **respiratory system,** by means of which the body receives oxygen and disposes of carbon dioxide to maintain its metabolic activities.

8. The **blood-vascular system,** including the circulating medium, the blood, together with the closed system of tubes through which it circulates.

9. The **nephric system,** by means of which the waste products of metabolism are eliminated from the body.

10. The **genital system.** This includes the organs in which the germ cells develop and by which they leave the body. These organs are unlike in the two sexes. As they are closely associated with the nephric organs, the two systems are sometimes spoken of together as the urogenital system.

The vertebrate classes. — The vertebrates are divided traditionally into five classes: fish, amphibia, reptiles, birds, and mammals. Recent classifications, which include not only living but fossil vertebrates, increase this number to eight. The system used in this book is a modification of that proposed by Gregory (1920).

1. **Ostracodermata.** This group includes the oldest representatives of the vertebrates, known only from fossils, and is entirely extinct at the present time. The skin was armored with plates of bone, for which reason the Ostracodermata are called the armored fish. Their relationship to the living classes is uncertain.

2. **Cyclostomata.** Here are included the lampreys and hagfish, forms which have a cartilaginous skeleton and naked skin. The mouth is round and lacks paired jaws. Paired appendages are also lacking. The Cyclostomata are called the round-mouthed fish.

3. **Chondrichthyes.** This group contains not only the modern elasmobranchs (sharks and rays) but also the bizarre chimæras and many extinct groups, found as fossils in very ancient rocks. They are the first fish with paired jaws and two pairs of appendages, the pectoral and pelvic fins. The skin is covered with thorn-like placoid scales, while the skeleton is cartilaginous. The members of the group are called the cartilage fish.

4. **Osteichthyes.** This group contains all the other fish, which are characterized by the partial or complete ossification of the skeleton and the possession of scales of different types, usually arranged like shingles. The members of this group are called the bony fish.

5. **Amphibia.** Including the salamanders and frogs of the present time as well as many ancient extinct groups, the amphibia are of particular interest as they form the connecting link between the classes that have been mentioned above and those that are to be discussed. Neither wholly terrestrial nor wholly

aquatic, they have been given a name that implies their intermediate habitat. They breathe by gills, by lungs, or through the skin, which is naked. The paired appendages terminate in (typically) five digits. The eggs are usually laid in water.

6. **Reptilia.** The reptiles include the turtles, lizards, snakes, and alligators, as well as a wealth of fossil forms. Respiration is exclusively by lungs, although many reptiles live in the water. The skin is covered with horny scales. The eggs are laid on land.

7. **Aves.** These are the birds. Horny scales are found on the legs (pelvic appendages) but the body is covered with feathers, as are the pectoral appendages, which are modified for flight. The birds resemble the reptiles in so many important anatomical characters that the two groups are often known collectively as the **Sauropsida.**

8. **Mammalia.** In this group the skin is typically covered with hair. The principal diagnostic character is that the newborn young are nourished by milk secreted in the breasts of the mother. Three sub-classes of living mammals are distinguished. In the **Prototheria,** eggs are laid and the young hatched out from them, as in the sauropsida. In the **Metatheria,** the young are born before the fœtal stage is completed, and are carried in a pouch, the **marsupium,** until able to obtain food for themselves. In the **Eutheria,** the young are retained in the uterus for a much longer period and obtain their nourishment from the maternal blood by means of a fœtal organ known as the **placenta.**

The super-classes. — From the standpoint of comparative anatomy and palæontology, the first four classes form one major group, the **Pisces** (used in the broadest sense of the word), including all the gill-breathing types in which the paired appendages are fins; the last four constitute a group, the **Tetrapoda,** in which the appendages usually terminate in five digits, and lungs are formed in development, whether or not they actually become the functional organs of respiration. From the embryological evidence, a slightly different method of grouping is of value. In this the Amphibia are removed from the Tetrapoda and included with the Pisces to form a group known as the **Anamniota,** characterized by the lack of any protection for the developing embryo other than the egg envelopes and shell, if one is formed. The Reptilia, Aves, and Mammalia, in which a protective em-

bryonic membrane, the **amnion,** is formed, are grouped together as the **Amniota.** These relationships are shown graphically in Table I.

TABLE I

Anatomical Groups	Classes	Embryological Groups
Pisces	1. Ostracodermata 2. Cyclostomata 3. Chondrichthyes 4. Osteichthyes	Anamniota
Tetrapoda	5. Amphibia	
	6. Reptilia 7. Aves 8. Mammalia	Amniota

C. PLAN OF THE BOOK

The present text is an introduction to the embryology of the vertebrates from the comparative standpoint. Although this subject is primarily morphological, it will be necessary, from time to time, to discuss observations, or the results of experiments, made upon invertebrate or even plant material, where such discussions will help in understanding vertebrate embryology. The book is divided into four main parts as described below.

Parts. — Part I, early embryology, includes the study of gametogenesis, fertilization, and the development of the embryo prior to the formation of the organ systems. The natural stages are considered in chronological order and from the comparative standpoint. **Part II, organogeny,** includes a study of the outlines of organ formation. The organ systems are taken up in the order of the germ layers from which their essential parts are derived. After a general discussion, the principal differences in organogeny between the frog, chick, and man are indicated. In **Part III, atlas of sections,** there are included figures and descriptions of particularly important sections through different parts of the bodies of vertebrate embryos. Three types have been selected, the frog, chick, and pig. The frog is selected not only because

of the ease with which laboratory material can be secured, but because it belongs to the important transitional group, Amphibia; because a larval stage occurs, and because the amount of yolk is not excessive. It serves as a type of the small-yolked anamniota. The chick has long been a favorite subject for the laboratory study of embryology. It serves as an example of development among the large-yolked sauropsida. The pig serves as an example of a mammalian embryo that can be obtained without difficulty. **Part IV** is a section on **microscopical technique,** the term applied to the methods by which embryos are collected, preserved, and prepared for detailed study. In this division of the text are included some directions as to the use of the microscope and its accessories and some suggestions as to the methods used in studying embryological material. Since our modern embryology depends so largely upon improvements in preparation and observation, it is hoped that every student will have an opportunity to make some of his own preparations. When, however, this is not feasible, a knowledge of how the laboratory material is prepared may assist in its interpretation.

Authorities. — In addition to the scientific journals which contain articles on vertebrate embryology, there are certain authorities which have been frequently consulted in the preparation of this book, and which should, if possible, be accessible to the student. References for supplementary reading are listed at the end of each chapter; below are given the books that the author has found indispensable.

1. Hertwig, O. 1906. Handbuch der vergleichenden und experimentallen Entwickelungslehre der Wirbeltiere. This monumental collection of monographs under the editorship of Hertwig remains the principal source for materials in the study of vertebrate embryology.

2. Korschelt and Heider. 1902–1910. Lehrbuch der vergleichenden Entwicklungsgeschichte der wirbellosen Tiere. Although primarily devoted to the invertebrates, this important manual includes discussions of the early development of the vertebrates.

3. Keibel, F. 1897–1923. Normentafeln zur Entwicklungsgeschichte der Wirbeltiere. This series of atlases, commenced under the editorship of Keibel, figures the natural stages of devel-

opment for many embryos, including those of the chick, pig, and man.

4. Duval, M. 1889. Atlas d'Embryologie. This is particularly valuable for the unsurpassed figures of sections of chick embryos which it contains.

5. Textbook of Embryology. Volume I, Invertebrates, by MacBride, E. W., 1914. Volume II, Vertebrates exclusive of Mammals, by Graham-Kerr, J., 1919. (Volume III, Mammals, not yet published.) These more recent treatises supplement the works mentioned above and must replace them for those who read no foreign languages.

6. Keibel and Mall. 1910–1912. Human Embryology. This great work, like Hertwig's, is a co-operative series of monographs.

7. Brachet, A. 1921. Traité d'Embryologie des Vertébrés. This is the most recent embryology embodying any original contributions and has been found most helpful in the preparation of the present text.

8. Wilson, E. B. 1925. The Cell in Development and Heredity, 3rd edition. This work is indispensable to the student of early embryology.

9. Lee, A. B. 1929. The Microtomist's Vade-mecum, 9th edition. An encyclopædic little volume that should be in every laboratory.

10. Gage, S. H. 1925. The Microscope, 14th edition. This should stand beside the Vade-mecum, on the laboratory shelf.

Summary. — Embryology is the study of the development of an individual. It is divided into the sub-sciences of comparative embryology and experimental embryology. Comparative embryology traces development through three periods: gametogenesis, in which the egg and sperm are formed; fertilization, in which the egg and sperm unite to form the zygote; and the period in which the embryo is formed. During the last period, the developing embryo passes through three stages: cleavage, in which the zygote divides into blastomeres; the stage in which the blastomeres become arranged into the germ layers; and organogeny, in which the organ systems are formed from the germ layers. The study of comparative embryology has given rise to the recapitulation theory which is one of the buttresses of the evolutionary hypothesis. Experimental embryology seeks to analyze the factors involved in development or heredity in action, which is regarded as an

epigenetic process. These factors are cell division, growth, molding, localization, and differentiation. The study of experimental embryology brings the zoölogist into close contact with the study of chemistry and physics and has resulted in the so-called mechanistic conception of vital processes.

A review of the vertebrates indicates briefly their systematic position, gives a diagnosis of the vertebrate body plan, mentions the major divisions, and lists the organ systems.

The plan of the text is to discuss the major phenomena of early embryology, the elements of organogeny common to all vertebrates, and to show by illustrative sections the principal embryonic structures of the frog, chick, and pig. It concludes with a section on the most useful methods of preparing and studying embryological material.

REFERENCES

Brachet, A. 1921. Traité d'Embryologie, Introduction.
Gregory, W. K. 1920. Bulletin of the American Museum of Natural History, XLII, 101.
Hertwig, O. 1906. Handbuch, Introduction.
Jenkinson, J. W. 1909. Experimental Embryology, Introduction.
1913. Vertebrate Embryology, Introduction.
Kellicott, W. E. 1913. General Embryology, Chapter I.
Morgan, T. H. 1927. Experimental Embryology, Introduction.
Wilson, E. B. 1925. The Cell, Introduction, pages 980, 1106–1118.

PART I

EARLY EMBRYOLOGY

CHAPTER II

THE GERM CELLS

The type of reproduction characteristic of the vertebrates is **bisexual.** The new individual originates from a germ formed by the union of two germ cells, or gametes, the larger of which is produced by the female parent and known as the **ovum,** while the smaller, produced by the male parent, is called the **spermatozoön.**

Among invertebrates, it is not uncommon for the ovum to develop independently of the spermatozoön, a phenomenon known as **parthenogenesis.** This has never been observed in the vertebrates except when induced by physical or chemical stimulation, **artificial parthenogenesis,** as in the case of the frog's egg.

Bisexuality usually implies that the two sexes are represented by different individuals, and this is the rule among vertebrates. Among the protochordates, we find that in some of the tunicates both eggs and sperm are produced by the same individual, which is therefore known as a **hermaphrodite.** Hermaphroditism, in the sense that both types of germ cells may be produced by the same individual, is a rare phenomenon among the vertebrates, and not typical of any species.

A. THE GAMETES

The gametes are atypical cells which differ widely in appearance. Before discussing the structure of the egg and sperm, it is advisable to review the structure of a generalized cell (Fig. 1).

The cell. — The familiar definition of a cell is " a mass of protoplasm, containing a **nucleus,** both of which have arisen by the division of the corresponding elements of a pre-existing cell." The term **protoplasm,** as generally used, includes the substance

of the cell both within and without the nucleus, so that it is customary to use the term **karyoplasm** for the nuclear protoplasm, and the word **cytoplasm** for the extra-nuclear protoplasm of the cell body.

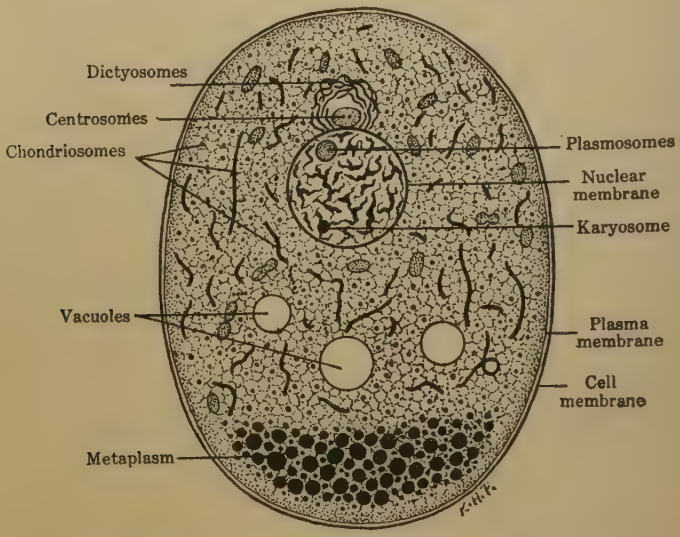

FIG. 1. — Diagram of a generalized cell. (After Wilson, "The Cell in Development and Heredity," copyright, 1925, by the Macmillan Co., modified.)

Protoplasm. — From the chemical standpoint, protoplasm is a complex of proteins, carbohydrates, lipoids, and inorganic salts, together with large amounts of water. The molecules are extremely large, complex, and changeable. Physically, protoplasm is classed as a complex colloidal system, ordinarily appearing as a liquid of considerable viscosity, but sometimes becoming transformed to a more solid consistency. This change is reversible. As nearly as can be observed, protoplasm is composed of a liquid basic substance, the **hyaloplasm,** in which are distributed more highly differentiated elements known as **formed bodies,** some of which are considered as living, others as lifeless (**deutoplasm**).

Formed bodies of the cytoplasm. — The cytoplasm contains many formed bodies which have assumed a considerable im-

portance in recent times. Of these cytoplasmic inclusions, the more important seem to be the **centrosomes, chondriosomes,** and **dictyosomes.** All these appear to have the power of independent growth and division. The centrosomes usually occur near the nucleus. They may be one or two in number and are particularly noticeable during cell division, in which they play an important rôle. They are small spherical bodies differing in size in the cells of different animals. The chondriosomes are small bodies scattered through the cytoplasm; they appear as small granules (mitochondria), rods, or spheres, are essentially of lipoid nature, are dissolved by ordinary fixing fluids, and are stained intra-vitally by a particular stain, Janus Green B. Their function is unknown but there are many theories as to their nature, of which the more important are: (1) that they are symbiotic bacteria; (2) that they are concerned with the formation of deutoplasm; and (3) that they are concerned with the mechanism of heredity. The dictyosomes also appear to be of lipoid nature. They appear as granules, curved rods, plates, or ring-like bodies. Often scattered throughout the cytoplasm, they are sometimes aggregated about the centrosome, in which case the aggregate is called the **Golgi apparatus.** Recent researches seem to indicate that the dictyosomes are concerned with the elaboration of intracellular secretions. The term **microsomes** is applied to small granules without reference to their function.

Deutoplasm. — Formed bodies that do not have the power of independent growth or division are classed together as the deutoplasm. Among these are **secretory granules, storage granules,** and **pigment granules.** Secretory granules are transitory structures which change in size and staining reactions until they finally dissolve to produce enzymes, mucin, or fat. Storage granules represent stages in the accumulation of food material such as yolk, oil, starch, etc. Pigment granules are a type of storage granules which together produce the color of the cell.

Plasma-membrane. — All cells are surrounded by a **plasma-membrane,** which is a part of the cytoplasm, differentiated by its physical consistency. It acts as a semi-permeable membrane and has an important rôle in the activities of the cell as influenced by the environment.

The true membrane. — Some cells form, in addition to the plasma-membrane, and external to it, a secondary envelope, the **true membrane.** This is now generally held to be a lifeless secretion product of the cytoplasm. In some tissues, such as cartilage and bone, the secretion of these envelopes is carried to such an extent that the cells become widely separated in a continuous matrix.

The nucleus. — The **cell nucleus** usually appears as a rounded mass of protoplasm, separated from the cytoplasm by a delicate **nuclear membrane** and including granules of a peculiar substance called **chromatin.** This type of nucleus is said to be **vesicular.** Two other conditions are recognized: the **massive** type, in which the chromatin is aggregated into a compact body; and the **diffuse** type, in which the chromatin is scattered throughout the entire cell as granules called **chromidia.** All these types are linked by intermediate conditions.

The karyoplasm. — The basic substance of the nucleus is comparable to the hyaloplasm of the cell body, and similar controversies have arisen as to whether its structure is essentially alveolar, fibrillar, or granular, with approximately the same result — the Scotch verdict, "not proven." The karyoplasm appears to consist of a liquid, the **nuclear sap,** in which are suspended (or dissolved) the particles of chromatin, which are classified according to their staining reactions. Those that are deeply colored by basic dyes are known as **basichromatin;** those that take up the acidic stains are called **oxychromatin.** However, it has been demonstrated that chromatin may change from a basophilic to an oxyphilic condition, or *vice versa*, possibly as a result of the amount of nucleic acid present.

The nucleoli. — Within the nucleus we are frequently able to distinguish small round bodies called **nucleoli.** These fall into two classes according to their staining reactions. The **plasmosomes** are oxyphilic, usually in the form of liquid drops. They appear to be distinct from the general framework of the nucleus. They have sometimes been considered centers for the elaboration of nuclear material, sometimes accumulations of waste products. The **karyosomes** are basophilic and contribute basichromatin in the process of cell division. Plasmosomes and karyosomes are occasionally associated in a common body, to which the name **amphinucleolus** is applied.

The nuclear membrane. — The membrane surrounding the nucleus is a thin film which is sometimes formed from the cytoplasm (inner cell membrane), and sometimes from the framework of the nucleus itself. It is generally considered a definite continuous structure, and is probably, like the plasma-membrane, semi-permeable in nature.

TABLE II

Parts of the cell

A. Cell body composed of cytoplasm.
  1. Hyaloplasm.
  2. Formed bodies.
    a. Protoplasmic.
      (1) Centrosomes.
      (2) Chondriosomes.
      (3) Dictyosomes (sometimes forming Golgi apparatus).
    b. Deutoplasmic.
      (1) Secretory granules.
      (2) Storage granules.
      (3) Pigment granules.
  3. Plasma-membrane.
  4. True membrane.

B. Nucleus composed of karyoplasm.
  1. Nuclear sap.
  2. Chromatin.
    a. Basichromatin.
    b. Oxychromatin.
  3. Nucleoli.
    a. Karyosomes (of basichromatin).
    b. Plasmosomes (of oxychromatin).
    c. Amphinucleoli (mixed).
  4. Nuclear membrane.

Cell types. — The cell that has just been described is a generalized one. Living cells vary in size, in form, in the cell structures present, and, above all, in function. An independent cell, such as a protozoön, which can perform all the functions peculiar to living things, is called a physiologically balanced cell. A cell that forms a part of the metazoan body is specialized for some particular function of the body and is dependent upon other cells for other functions. Such a cell is said to be physiologically unbalanced. On the boundary line between these two classes we find the germ cells, atypical and usually incapable of further development by themselves, differing widely between the male and female products, and yet so correlated that in the union

between the egg and sperm are brought together the materials which give rise to a new individual.

The spermatozoön. — The male germ cell is a very minute, flagellate, and highly motile cell. The size range in length is from 20 microns in the crocodile to over 2 millimeters in the amphibian Discoglossus. The general shape, that of a tadpole with an excessively elongate tail, is similar throughout the vertebrate group; but even in these tiny cells so many differences of structure are apparent that it has been said that the species of any vertebrate could be identified from a study of the spermatozoa.

The spermatozoön (Fig. 2) is divided externally into the **head,** which is morphologically the anterior part of the cell, and the **tail,** which comprises the greater part of the cell so far as length is concerned. The head contains the nucleus which is of the massive type and extremely basophilic. Here also is found the **acrosome,** usually at the apical end, a body derived from the dictyosomes and probably concerned with the elaboration of some secretion involved in fertilization. The head is surrounded by a delicate cytoplasmic **sheath,** which is probably a plasma-membrane; it sometimes contains **fibrillæ** which wind around the nucleus in a spiral fashion. The fibrillæ have been regarded as skeletal structures; there is some evidence to indicate that they are derived from chondriosomes.

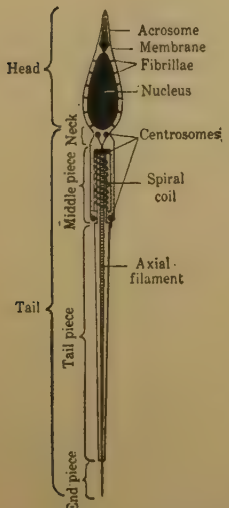

FIG. 2.—Diagram of a spermatozoön. (After Wilson, "The Cell in Development and Heredity," copyright, 1925, by the Macmillan Co., modified.)

The tail is divided into four typical regions: **neck, middle-piece, main-piece,** and **end-piece,** not all of which are to be distinguished in every type of sperm. The neck is a constricted region next to the head, which contains one or more centrosomes or structures derived from them. These are sometimes pushed up into the base of the nucleus so that no neck is distinguishable. From these **proximal** centrosomes the **axial filament** of the tail takes its origin. No material of dictyosomal or chondriosomal origin has

been identified in the neck. It is surrounded by a plasma-membrane continuous with that of the head. The middle-piece also contains a centrosome. This **distal** centrosome is divided into two portions which mark, respectively, the anterior and posterior limits of this region. The axial filament runs through these bodies and is surrounded by a **spiral coil** of chondriosomal material. A plasma-membrane is present. The main-piece, by far the longest portion of the tail, is composed of the axial filament, which consists of one or more contractile fibrillæ by means of which the tail is moved, and its sheath. There is some controversy as to whether the latter is chondriosomal in origin, a plasma-membrane, or a derivative of the axial filament. The end-piece of the tail is the region in which the naked axial filament projects beyond the limits of the sheath.

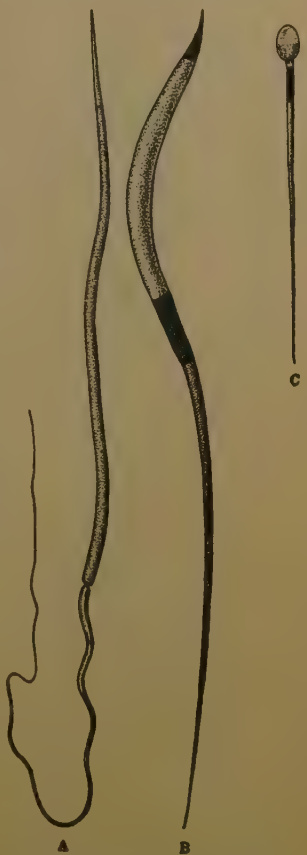

Fig. 3. — Types of spermatozoa. A, the frog. B, the fowl. C, human. (After Retzius, redrawn to be of same magnification.)

The frog. — The spermatozoa of the frog (Fig. 3*A*) have an elongate head and sharply pointed acrosome. The neck is indistinguishable, but two **basal bodies** lying side by side at the base of the nucleus represent the proximal centrosome. The length of the sperm is about 0.1 mm.

The chick. — The sperm of the fowl (Fig. 3*B*) has a long cylindrical head with a pointed acrosome; the neck is short, and the middle-piece elongate with a spiral envelope. The length of the sperm is approximately 0.08 mm.

Man. — The human spermatozoön (Fig. 3*C*) has a head shaped like a compressed ovoid, with the acrosome, which is flattened

at the apex into a sharp edge, fitting over the anterior end like a cap. A constricted neck contains the proximal centrosome, which is flattened out into a **basal disc.** The middle-piece contains a spiral chondriosomal sheath around the axial filament. The length of the sperm is 0.055 mm.

Sperm dimorphism. — Even in the same individual, two or more classes of sperm may be formed. Pathological differences are due to the occasional formation of dwarf or giant spermatozoa, and sometimes to the appearance of conjugate sperm in which the heads are united. **Sexual dimorphism,** in which two classes of sperm, respectively male-producing and female-producing, are formed, is characteristic of all vertebrates except birds. This phenomenon will be discussed in a later section (see pages 50, 51).

The ovum. — The ovum (Fig. 4) is a giant cell, protected by envelopes of various kinds, containing reserve food material and without power of locomotion. The size range in diameter among living vertebrates is from 0.06 mm. in the mouse to 85.0 mm. in the ostrich (exclusive of albumen and shell). Larger eggs are recorded among the extinct races of reptiles and birds. The fossil egg shells of the extinct bird Æpyornis measure 13 inches by 9, and have a capacity of approximately 2 gallons. The shape of the ovum is usually spherical, although sometimes ellipsoid or oval.

Fig. 4. — The ovum of the cat. (After Dahlgren and Kepner, "Principles of Animal Histology," copyright by the Macmillan Co., 1908, modified.)

Types. — The size of the egg is conditioned largely by the amount of yolk present. We distinguish three classes of eggs, based on the amount of the yolk. An **oligolecithal** egg is one in which the amount of yolk is very small, as in Amphioxus (Fig. 5*A*) and, at the other end of the vertebrate series, the placental mammals (Fig. 5*D*). In these eggs the yolk globules are small and approximately equally distributed

throughout the cytoplasm, a condition sometimes known as **isolecithal.**

If the amount of yolk is quite noticeable, and it is aggregated principally in one hemisphere of the egg, as in the cyclostomes, some osteichthyes, and the amphibians, the egg is called **lecithal** (Fig. 5*B*). The distribution of the yolk in the lower hemisphere

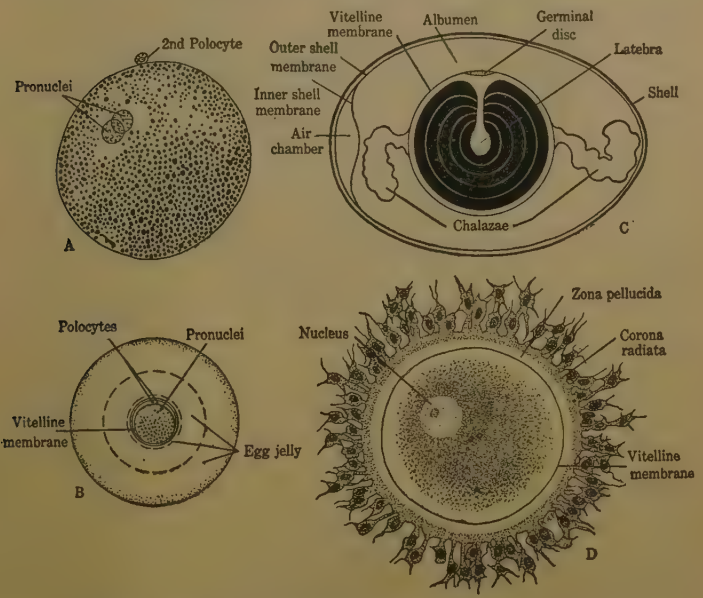

FIG. 5. — Types of eggs. A, Amphioxus (after Cerfontaine). B, the frog (after Schulze). C, the hen (after Lillie). D, human (after Waldeyer).

is due to the fact that it possesses a higher specific gravity than the cytoplasm; this type of distribution is known as **telolecithal.**

Finally, in eggs where the amount of yolk is so enormous that the clear cytoplasm and nucleus form only a small disc on a great globe of yolk, as in the chondrichthyes, some osteichthyes and the sauropsida, the type is known as **macrolecithal** (Fig. 5*C*). The distribution is an exaggerated form of the telolecithal condition.

Cytoplasmic structures. — In the ovum, the cytoplasm and its contained structures are more conspicuous than in the spermatozoön. As has been indicated, the yolk forms a large part of the egg bulk. This substance contains the principal foodstuffs for the developing embryo: fats, proteins, and sometimes carbohydrates, inorganic salts, and water. The yolk takes the form of spheres, ovoids, or discs. The staining reaction is generally basophilic. As the yolk is heavier than cytoplasm, it tends to collect in the lower hemisphere if present in considerable quantity. Accordingly, a line drawn through the centers of the two hemispheres will form an **egg axis,** about which the egg is apparently radially symmetrical. The center of the upper hemisphere is called the **apical pole;** that of the lower hemisphere, the **abapical pole.** The **polarity** of the egg seems to be determined before its escape from the ovary.

The color of the egg cytoplasm is due to granules or droplets of pigment which are sometimes diffuse and sometimes arranged in patterns at the periphery of the egg or around the nucleus.

The centrosomes are present in the egg before maturation, but cannot be identified thereafter. Chondriosomes and dictyosomes have also been identified in the cytoplasm. These will be discussed at greater length in connection with the development of the ovum.

The nucleus. — The study of the egg nucleus is complicated by the fact that it is undergoing the changes of maturation, **meiosis.** Prior to this it is a large vesicular structure with one or more nucleoli. During this period the chromatin undergoes a progressive change from the basophilic to the oxyphilic condition. The nucleoli may appear as plasmosomes, karyosomes or amphinucleoli. The position of the nucleus varies with the type of egg. It is central in oligolecithal eggs, eccentric in lecithal, and in the center of the protoplasmic disc in macrolecithal eggs, thus obeying Hertwig's law, which states that the nucleus lies in the center of its field of activity, the cytoplasm (see page 61).

Egg envelopes. — The ovum usually possesses, in addition to the plasma-membrane, a variety of protective envelopes, which are divided into three classes according to their mode of formation.

The **primary** envelopes are those formed by the egg itself, such as the delicate **vitelline membrane.** The **secondary** envelopes are those formed by the follicle cells which immediately surround the egg in the ovary. A good example is the so-called " **chorion** " of one of the cyclostomes, Myxine (Fig. 6). It is usually quite difficult to distinguish primary from secondary envelopes, and it is probable that many vitelline membranes are compound in origin. In those vertebrates in which fertilization is external, such as the cyclostomes and most osteichthyes, the primary

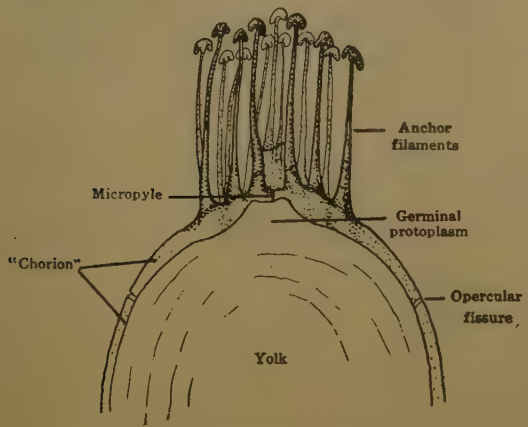

FIG. 6. — The egg of Myxine to show "chorion." (After Dean.)

and secondary envelopes are often perforated by openings called **micropyles** through which the sperm may have access to the egg. The **tertiary** envelopes include all those formed by the walls of the oviduct during the passage of the egg. These include the egg albumen, shell membranes, and shells of reptiles, birds and the egg-laying mammals; the egg capsules of the chondrichthyes; and the egg jelly of the amphibia and many osteichthyes. These envelopes are not formed until after fertilization except in the case of the egg jelly, and this does not attain its final thickness until after the entrance of the sperm, when it swells by the absorption of water.

Amphioxus. — The egg of Amphioxus is a good example of an oligolecithal egg (Fig. 5*A*). The yolk is distributed almost uni-

formly throughout the cytoplasm, except for a cone-shaped area about the nucleus. A vitelline membrane is present.

The frog. — The egg of the frog is lecithal (Fig. 5*B*). The yolk is concentrated in the abapical hemisphere. The nucleus lies in the center of the apical hemisphere. The dark pigment of this upper hemisphere of the egg is caused by granules of **melanin** in the cortical layer. Pigment is also found around the nucleus. A vitelline membrane is present, as are also from one to three layers of egg jelly. The diameter varies in different species, averaging about 1.6 mm.

The chick. — The hen's egg (Fig. 5*C*) is macrolecithal. The cytoplasm, with the nucleus in its center, lies as a small disc, the **germinal disc,** upon the great mass of yolk. This is arranged in concentric layers of yellow and white material around a central mass of white yolk, called the **latebra,** which extends upward as a stalk of white yolk, known as the **isthmus** (**of Pander**). The yolk and germinal disc are surrounded by a delicate vitelline membrane. This in turn is surrounded by the albumen, a viscous tertiary membrane twisted spirally about the egg from left to right, starting from the broad end of the egg. The albumen next to the vitelline membrane is denser than the rest and is prolonged into two spirally twisted cords, the **chalazæ,** one at either end of the egg. The albumen is in turn surrounded by two parchment-like shell membranes, of which the inner one is the thinner. These two are separated at the blunt end of the egg, thus forming the **air chamber.** The egg shell is a calcareous deposit upon the outer shell membrane. Its color is due to bile pigments of the hen. The germinal disc is about 4 mm. in diameter, the yolk about 40 mm. The size of the egg as a whole varies largely.

Man. — The human egg (Fig. 5*D*) is oligolecithal. The yolk granules are concentrated about the nucleus which is slightly eccentric. A vitelline membrane and **zona pellucida,** also a primary envelope, are present. When the egg is expelled from the ovary a layer of follicle cells usually persists about it, to form the **corona radiata,** which later degenerates. The diameter of the egg is about 0.2 mm.

Egg dimorphism. — Pathological differences are observed in eggs as well as in sperm. Giant and dwarf eggs are well known, as are such anomalies as the inclusion of two or more eggs in a

single shell. **Sexual dimorphism,** in which two classes of eggs, respectively male-producing and female-producing, are formed, is a phenomenon characteristic of the birds. This statement is made rather as a deduction from the nature of heredity in this group than because of any morphological observations.

Comparison of the egg and the sperm. — Both gametes are morphologically complete cells. Each has a nucleus and cytoplasm with representatives of the centrosomes, chondriosomes, and dictyosomes. Each has a plasma-membrane. Yet neither is capable of independent, continued existence, for physiologically they are unbalanced. The egg is large, inert, and contains a vast store of deutoplasm, is protected by egg envelopes, and has lost the power of continued division. The sperm is small, highly motile, contains no deutoplasm and little cytoplasm, is devoid of protective membranes, and in itself has lost the power of continued division. We shall now turn to the study of the development of the germ cells and see how the structural differences arise.

TABLE III

Comparison of egg and sperm

Ovum	Generalized Cell	Spermatozoön
Large amount	Cytoplasm	Small amount
One, disappears	Centrosomes	Two, retained
Diffuse	Chondriosomes	Spiral sheath
Diffuse	Dictyosomes	Acrosome
Present	Plasma-membrane	Present
Vitelline membrane	True membrane	Absent
Vesicular	Nucleus	Massive
Both types	Nucleoli	Indistinguishable
Present	Nuclear membrane	Present
.....	.....	.....
Large	Size	Small
Quiescent	Movement	Swims actively
None	Motile organs	Flagellum
Egg envelopes	Protection	None

B. GAMETOGENESIS AND FERTILIZATION

Gametogenesis, by a curious paradox, is not only a part of the development of an individual, inasmuch as it is the study of the development of the gametes whose union produces the indi-

vidual, but it is also a part of the development of the parents within whose bodies the gametes are formed.

The germ-plasm. — Our account of gametogenesis, then, begins with the fertilized egg of the preceding generation, a parental zygote. From this cell we should, theoretically, be able to trace, cell division after cell division, the lineage of the ultimate gametes, the **germ-plasm,** as distinguished from the history of all the other cells of the body, the **somatoplasm** (Fig. 7). This

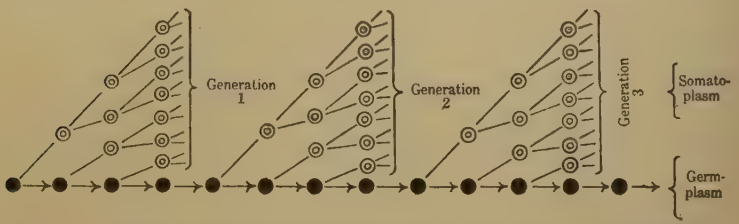

FIG. 7. — Diagram to illustrate relationship between germ-plasm and somatoplasm. (After Wilson, "The Cell in Development and Heredity," copyright, 1925, by the Macmillan Co., modified.)

conception of the purity of the germ-plasm is due to Weismann and has pointed the way to much experimental work.

The germ line. — In some few invertebrate animals, e.g., Ascaris, it has been possible to trace the history of the germ cells from the first division of the fertilized egg. The series of cells arising from this succession of divisions is called the **germ line** (Fig. 8). In Ascaris, one of the first two cells produced by the dividing zygote produces only soma-cells; the other, in the succeeding division, gives rise to a soma-cell and one like itself. The first cell then is a soma-cell; the second, a **stem-cell.** The stem-cells, after several divisions in which both soma-cells and stem-cells are produced, eventually cease the formation of soma-cells. Their products are now known as the **primordial germ cells.** These are the first stages in the germ line that are generally recognizable. In the vertebrates they sometimes appear in the endoderm of the midgut and migrate from that position, by way of the dorsal mesentery, to the dorsal wall of the cœlom. In other cases the primordial germ cells make their first appearance in the cœlomic roof. In either case they cease division for a time and undergo changes

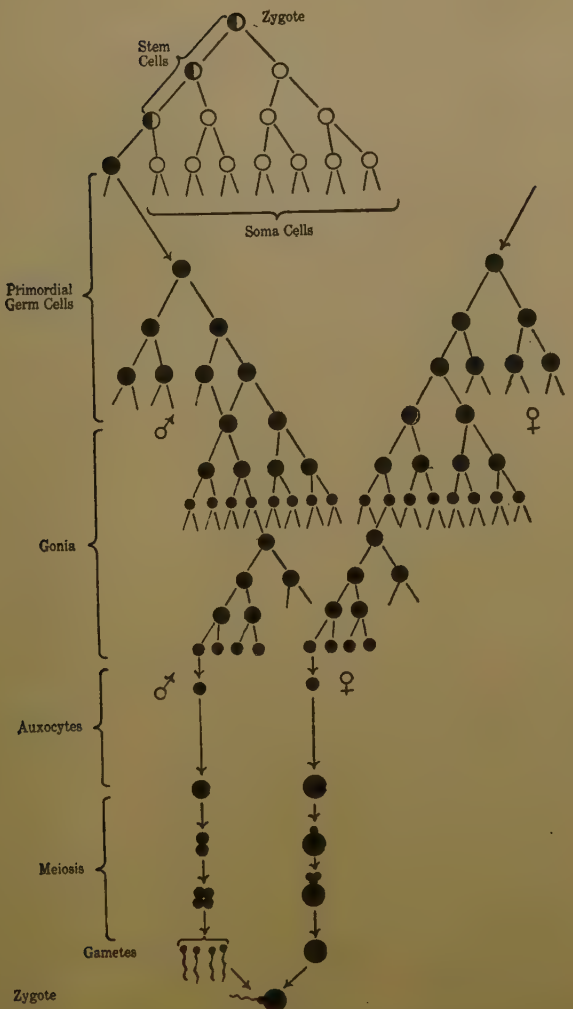

FIG. 8. — Diagram of the germ line, male on left, female on right. (After Wilson, "The Cell in Development and Heredity," copyright, 1925, by the Macmillan Co., modified.)

TABLE IV

The germ line

MALE	INDIFFERENT	FEMALE
	The zygote	
	Period of Differentiation Stem-cells and soma-cells	
	Period of Migration Primordial germ cells	
	Period of Multiplication	
Spermatogonia	Gonia	Oögonia
	Period of Growth	
Spermatocytes	Auxocytes	Oöcytes
	Period of Maturation	
One primary spermatocyte		One primary oöcyte
	First Meiotic Division	
Two secondary spermatocytes		Two secondary oöcytes = one ovum and one polocyte
	Second Meiotic Division	
Four spermatids		Four oötids = one ovum and three polocytes
Period of Transformation Four spermatozoa		
	The zygote	

in structure, after which they are known as **gonia.** These gonia aggregate to form germ-glands, or **gonads.** At first the sex of the gonia is not distinguishable; but later sex differences appear, after which the gonia of the male are known as **spermatogonia** and the male gonad as the **testis,** while the gonia of the female are called **oögonia,** and the female gonad the **ovary.** The gonia enter a period of rapid cell division, during which some of the daughter cells become nurse cells, such as the **follicle cells** of the ovary or the **sustentacular cells** of the testis. The gamete-producing gonia, however, enter upon a period of growth and internal change, at the end of which they are known as **auxocytes** (Fig. 9). Male auxocytes are designated as **spermatocytes;** female auxocytes, as **oöcytes.** These become mature by two divisions, the meiotic divisions, which are of great theoretical importance (see page 43). The later history of the auxocytes differs in the two sexes.

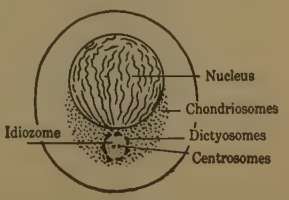

FIG. 9. — Diagram of an early auxocyte. (After Wilson, "The Cell in Development and Heredity," copyright, 1925, by the Macmillan Co.)

Spermatogenesis. — The male auxocyte, or **primary spermatocyte,** is a large cell containing a large vesicular nucleus, more or less eccentric. Near the nucleus, and in the center of the thicker layer of cytoplasm surrounding it, one or two centrosomes are embedded in a clear substance known as the **sphere substance.** This compound body is known as the **idiozome,** and with it are often associated the dictyosomes, sometimes so closely connected as to form an investing reticulum or even a shell. Around the idiozome are also grouped the chondriosomes to form a cap which sometimes includes the nucleus as well.

The primary spermatocyte divides, giving rise to two **secondary spermatocytes,** which divide again, often without intermission, each forming two **spermatids.** The four spermatids thus produced from the primary spermatocyte become transformed into the **spermatozoa.**

The spermatid. — During the two meiotic divisions mentioned above, the chromatin of the spermatocyte nucleus becomes dis-

tributed to the spermatids, which differ from each other in the quality (sometimes also quantity) of the chromatin received. The details will be discussed later (page 48). The centrosomes divide at each cell division so that each spermatid has a centrosome derived from the centrosome of the spermatocyte. The dictyosomes, each with a small amount of sphere substance, are segregated among the four spermatids in each of which they aggregate to form an **acroblast.** The chondriosomes are divided with almost exact evenness among the spermatids, in each of which they assemble to form a **paranucleus.** A plasma-membrane is present.

The spermatozoön. — In the transformation of the spermatid into the spermatozoön (Fig. 10), the nucleus, having previously

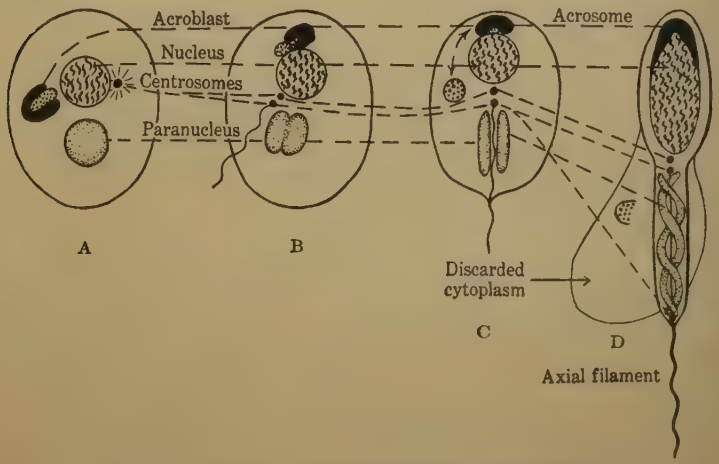

Fig. 10. — Diagram to show transformation of spermatid (A) into spermatozoön (D).

extruded a large amount of oxychromatin, condenses into a basophilic mass which elongates into its final shape. The centrosome divides, and the two centrosomes take up a position which marks the posterior end of the future spermatozoön, one centrosome lying against the nucleus, the other posterior to the first. The paranucleus also takes a posterior position, while the acroblast moves around the nucleus to the anterior end. The greater part of the cytoplasm is sloughed off.

The proximal centrosome remains in that part of the spermatozoön that will become the neck. The distal one divides into two portions, of which one remains in place while the other moves backward until it occupies a position which marks the posterior end of the middle-piece. During this migration the axial filament grows out in a posterior direction, while the moving part of the distal centrosome flattens out into a disc and separates into an inner basal body, attached to the axial filament, and an outer ring. The inner basal body then migrates forward to join the portion of the distal centrosome which remained in place, so that the axial filament now extends the length of the middle-piece. The paranucleus gives rise to the spiral envelope in this region. The axial filament carries with it part of the plasma-membrane as it grows back to form the main-piece of the tail, while the terminal portion of the filament which has grown out of the cell becomes the end-piece.

At the anterior end of the sperm, the acroblast divides into two portions, of which one is sloughed off with the cytoplasm while the other becomes the acrosome.

Oögenesis. — The female auxocyte, or **primary oöcyte,** is very like the primary spermatocyte. But the period of growth prior

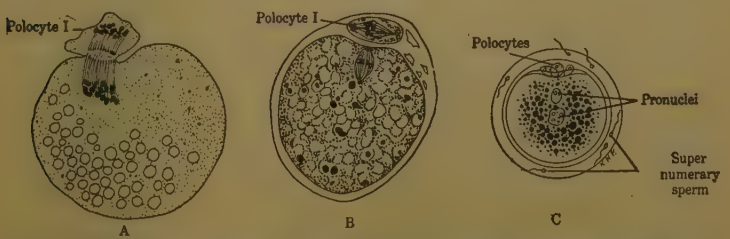

FIG. 11. — Stages in oögenesis. A, first meiotic division in guinea pig (after Lams). B, second meiotic division in bat (after Van der Stricht). C, fertilized egg of bat with three polocytes (after Van Beneden).

to the meiotic divisions is prolonged while large amounts of deutoplasm are formed. The nature of the meiotic divisions (Fig. 11) also is different, for the first, instead of giving rise to two **secondary oöcytes** of equal size, produces one very large cell and one excessively minute one. In the second meiotic division, the large secondary oöcyte again divides into one large cell and

one tiny cell, while the small secondary oöcyte may or may not divide into two equal cells. In this way four (or three, if the smaller secondary oöcyte fails to divide) **oötids** are produced, of which the single large cell is the **ovum,** while the smaller ones are known as **polocytes.** The latter are incapable of fertilization, and degenerate. As they are formed at the apical pole of the egg they are often called the polar bodies and are of practical value in orienting the egg.

We shall defer to a later section (page 43) an account of the details of the meiotic divisions, remarking here only that the principal nuclear result is that the chromatin is again sorted out into unlike aggregates, of which the egg and one polocyte differ from the remaining polocytes in the constitution of their respective nuclei. The centrosomes are often difficult to find in the primary oöcyte and have not been found in the ovum. The idiozome and the mass of chondriosomes are often associated in a **yolk-nucleus complex.** During the meiotic divisions a large amount of nuclear material is cast out into the cytoplasm. It has been suggested that the dictyosomes and chondriosomes might receive from the nucleus certain enzymes, and transport them to the different regions of the cytosome where the deutoplasm is elaborated.

Unlike the male gametes, whose maturation is completed within the testis, the oöcytes do not undergo their first meiotic division until after leaving the ovary; and, in the case of the vertebrates at least, the second division is not completed until after the entrance of the spermatozoön.

Fertilization. — This term is applied to the process by which a spermatozoön and an ovum unite, to form the **zygote,** or fertilized egg. Although by far the shortest period of embryology, it is perhaps the most important, for during this space of time the new individual takes its origin. We distinguish three stages in fertilization: (1) the **" attraction "** of the sperm by the egg, (2) the **" penetration "** of the egg by the sperm, and (3) the **conjugation** of the egg and sperm nuclei in the egg (Fig. 12).

Attraction. — Spermatozoa are usually immobile in the testis and the ductus deferens but become active upon entering the medium in which fertilization takes place. Under normal conditions the spermatozoa space themselves equidistantly in this

medium, but in the presence of the egg they tend to aggregate around it. This phenomenon was formerly explained by the assumption that the egg extruded a substance which attracted the sperm. It seems more probable that it is due to a " trap action," as a result of the fact that the sperm tends to remain in contact with a solid object, or an acid. Lillie suggests that a substance, **" fertilizin,"** is secreted by the egg and causes temporary reversible adhesion of the spermatozoa, a phenomenon known as **" agglutination."** The meeting of the egg and sperm is

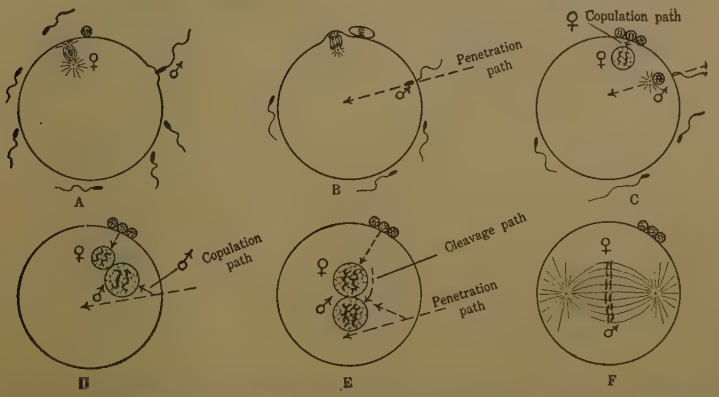

FIG. 12. — Diagram of fertilization. A, sperm entering egg. B, sperm engulfed. C, rotation of sperm head. D, pronuclei moving towards point of copulation. E, pronuclei on cleavage path. F, first cleavage.

initiated by neither but is due to a complex interaction involving activation, aggregation, and agglutination.

Penetration. — It was formerly supposed that the sperm bored its way into the egg, but more recent studies tend to show that it is drawn through the cell membrane by some unidentified physical factor. In some cases the sperm enters through the micropyle; in others the point of entrance is indeterminate, except in telolecithal eggs where the sperm enters the apical hemisphere. The egg, upon contact with the sperm, undergoes changes in the cortical region. A plasma-membrane is separated from the surface of the egg by the secretion of a jelly-like liquid. It was formerly held that the function of this **fertilization membrane** was to prevent the entrance of supernumerary spermatozoa,

polyspermy, but experiments have shown that after the entrance of the sperm this membrane may be removed without other sperm entering. Furthermore, there is a rush of cortical cytoplasm toward the point at which the sperm touches the egg, sometimes forming a protuberance known as the **entrance cone,** into which the sperm enters or is engulfed. By the retraction of the entrance cone, the sperm head, and sometimes the tail, is drawn into the egg (Fig. 12*A*).

Conjugation. — After the second meiotic division, which does not take place in vertebrates until after the entrance of the sperm, the nucleus of the ovum, or **female pronucleus,** is near the periphery at the apical pole, while the nucleus of the spermatozoön, or **male pronucleus,** is near the periphery at the point of penetration. The sperm head rotates 180°, so that the male pronucleus now lies distal to the middle-piece containing the centrosome. The two pronuclei now come together (Fig. 13) by a route which

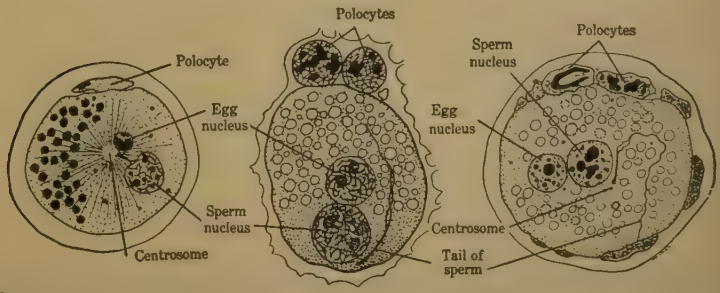

FIG. 13. — Fertilization of the egg of the guinea pig. Three stages following that represented in Fig. 11A. (After Lams.)

may be analyzed into the following components: (1) the sperm **penetration path** (Fig. 12*B*), which is usually the radius of the egg from the point at which the sperm entered; (2) the **sperm copulation path** (Fig. 12*D*), which is directed toward the point at which the pronuclei will meet and is often at a considerable angle to the penetration path; (3) the **egg copulation path** (Fig. 12*C*, *D*), along which the female pronculeus moves toward the meeting point; and (4) the **cleavage path** (Fig. 12*E*), along which the united pronuclei move to their final position on the egg axis, slightly nearer the apical pole. The two pronuclei do not

actually fuse, but each contributes a set of chromosomes in the ensuing division of the zygote. The centrosome of the egg disappears after the second meiotic division. The preponderance of evidence indicates that the centrosome of the zygote is either the sperm centrosome or one formed *de novo* near the middle-piece. This centrosome, however formed, divides as the pronuclei approach each other and supplies the centrosomes for the first division of the zygote. The chondriosomes of the sperm break up and scatter through the egg cytoplasm until they are indistinguishable from the maternal chondriosomes. The later history of the acrosome is unknown; it has been suggested by Bowen, however, that it may be the bearer of an enzyme concerned with the activation of the egg.

Polyspermy. — Among the vertebrates with macrolecithal eggs, it is the rule for many spermatozoa to enter the egg. Of these, only one supplies a male pronucleus to conjugate with the female pronucleus. The other or supernumerary sperm persist and may divide for a time but ultimately degenerate without contributing to the further development of the embryo.

Parthenogenesis. — This phenomenon is unknown among the vertebrates. Loeb, however, induced artificial parthenogenesis in the frog's egg by slight punctures with a finely pointed glass needle. More than twenty adults were reared in this manner from many thousand eggs which had been so treated. Of these, fifteen were male and three female, the others doubtful.

The frog. — The fertilization of the frog's egg is external, but the sperm are brought into close proximity to the eggs during the sexual embrace, or **amplexus.** During the breeding season the males clasp the females with the fore-legs, at which time the germ cells of each are extruded. The sperm make their way through the egg jelly before this envelope has attained its final thickness. The entire spermatozoön enters the egg, usually within 40° of the apical pole. The vitelline membrane is elevated as the fertilization membrane, leaving a space within which the egg may rotate. The second meiotic division then occurs, followed by the conjugation of the pronuclei. The penetration and sperm copulation paths are marked by a trail of pigment dragged in with the sperm head. A single spermatozoön alone enters the egg. Immediately upon fertilization the cortical

cytoplasm of the egg rushes toward the point of penetration, carrying with it the black pigment, melanin, of the apical hemisphere. Upon the side of the egg opposite the point of penetration, there appears a crescent-shaped area in which the pigment is less dense and which is, therefore, known as the **grey crescent** (Fig. 14).

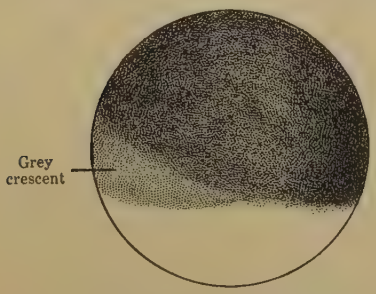

Fig. 14. — Frog's egg after fertilization.

The chick. — In the case of the fowl, fertilization is internal. The sperm, introduced into the cloaca of the female during copulation, make their way to the upper end of the oviduct, where fertilization takes place. Five or six spermatozoa enter the germinal disc, where they remain inactive until after the second meiotic division, when one of them moves inward until it comes in contact with the female pronucleus which has itself moved downward from the surface of the germinal disc. The supernumerary sperm move outward to the border of the disc, where, after a few divisions, they degenerate. The fertilized egg then moves slowly down the oviduct while the tertiary envelopes are forming about it.

Man. — Fertilization is internal and occurs at the upper end of the oviduct (Fallopian tube). Further details are lacking, as no direct observations have been recorded.

C. THE GAMETES AND HEREDITY

Particular importance attaches itself to the germ cells, since these are the material connection between parent and offspring, and through them alone can individual characters be transmitted. As has been pointed out, the germ cells become mature following two divisions of the auxocyte known as the meiotic divisions. But before proceeding to a study of these, it will first be necessary to examine the phenomena of the somatic type of division, characteristic of all other cells.

Mitosis. — Some few types of cells divide directly by a constriction of the cytoplasm following a similar division of the

nucleus. This phenomenon is known as **amitosis** and is comparatively unimportant among the vertebrates. The usual method is indirect and is characterized by the appearance within the nucleus of bodies of chromatin called **chromosomes,** each of which divides prior to the division of the nucleus and cytoplasm. This type of division is called **mitosis.** The phenomena of mitosis may be separated into two groups: (1) **karyokinesis,** which has to deal with the division of the nucleus; and (2) **cytokinesis,** or the division of the cell body.

Karyokinesis. — For convenience in description, the process of mitosis is divided into four phases based on the behavior of the chromosomes (Fig. 15).

In the first of these, the **prophase,** the chromatin is rearranged into a series of thin, convoluted threads, each apparently composed of a linear series of chromomeres. These threads, which may be separate from the beginning, or may segment from a continuous **spireme,** are the chromosomes in the **leptotene** stage (Fig. 15*B*). The threads now become shorter and thicker, the convolutions disappear, and the chromosomes are in the **pachytene** stage (Fig. 15*C*). The thick chromosomes now undergo a longitudinal split which brings them into the **diplotene** stage (Fig. 15*D*). There is some reason to believe that this split is initiated during the leptotene stage. After the formation of the chromosomes the nuclear membrane disappears.

In the **metaphase** (Fig. 15*E, F*) which follows, the chromosomes are arranged in a single plane across the equator of the nucleus, which is called the **equatorial plate.** The division planes of the chromosomes lie in this plate, while the chromosomes themselves have often condensed still further to form straight rods, loops, hooks, or even spheres. With this condensation the chromosomes acquire their greatest staining capacity and the division planes are more apparent. In some cases the chromosomes are arranged in pairs upon the equatorial plate.

In the succeeding **anaphase** (Fig. 15*G, H*) each chromosome divides longitudinally, so that two sets of homologous chromosomes are formed. Each set then moves toward one pole of the nucleus.

During the final stage, the **telophase** (Fig. 15*I*), each of these sets of chromosomes give rise to a new nucleus by a process

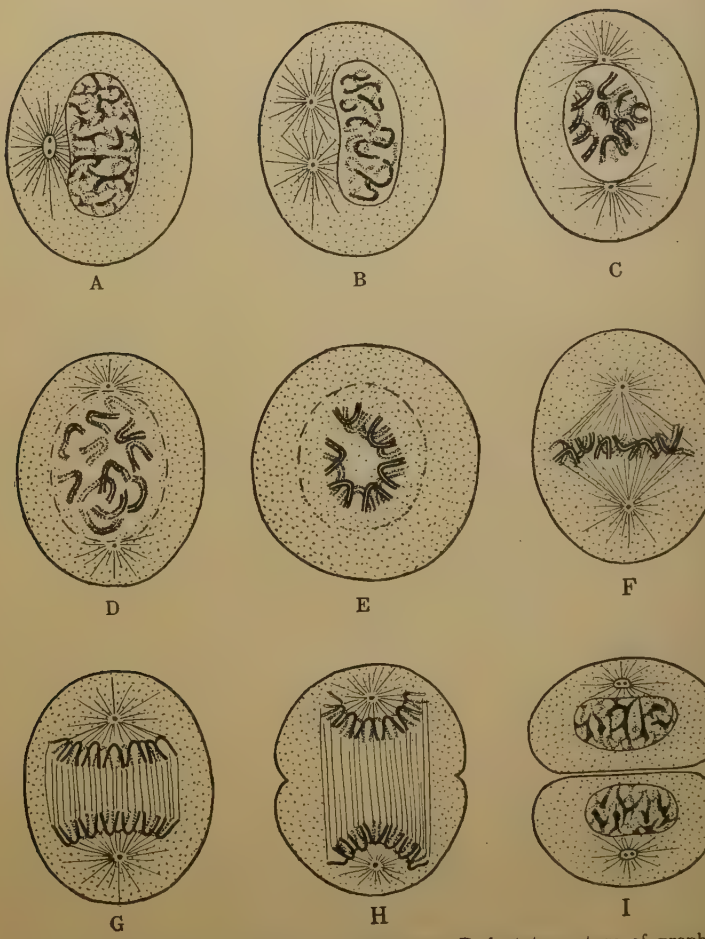

FIG. 15. — Diagram of mitosis. A, interphase. B, leptotene stage of prophase. C, pachytene stage of prophase. D, diplotene stage of prophase. E, metaphase, transverse section to show equatorial plate. F, metaphase, side view. G, early anaphase. H, late anaphase. I, two daughter cells in interphase. (After Corning.)

of reconstruction in which the chromosomes are resolved into their constituent chromomeres and a new nuclear membrane is formed. At this time the nucleoli are re-formed, for the karyosomes were utilized in the formation of the chromosomes at the beginning of the prophase, and the plasmosomes were cast out when the nuclear membrane was dissolved.

At times when cell division is proceeding rapidly it is customary to use the term **interphase** for the condition of the nucleus between divisions (Fig. 15*A*). At other times the expression "**resting nucleus**" is used, although the term is evidently malapropos.

Cytokinesis. — From the centrosome originates the **achromatic figure,** consisting, at its maximum period of development, of two centrosomes between which extends a fusiform mass of clear protoplasm known as the **spindle.** About each centrosome is a spherical mass known as the **aster,** in which radiating lines of microsomes appear. In prepared material both asters and spindle appear to contain numerous fibrillæ, **aster fibers** and **spindle fibers** respectively. Two types of spindle fibers are described, the first, or **centrosomal** fibers, extending from pole to pole of the spindle, while the second type, or **chromosomal** fibers extend from either pole to the chromosomes.

In the prophase, the centrosome, if it has not already divided during the preceding telophase, divides. The two centrosomes separate while a small aster develops about each and a spindle forms between them. Both spindle and asters increase in size until the length of the spindle is greater than the diameter of the nucleus. The nuclear wall dissolves. In the metaphase the chromosomes are drawn into the spindle, across the equator of which they assume their definite position. In the anaphase, the chromosomal fibers appear to shorten and thicken as the daughter chromosomes move toward their respective poles. In the telophase, spindle and asters disappear. Each centrosome is now associated with one of the daughter nuclei. Sometimes each divides in anticipation of the coming cell division. The chondriosomes usually divide *en masse* (Fig. 16*A*). This division of the chondriosomes is approximately an equal one, and there is some reason to believe that the individual chondriosomes divide during mitosis or just prior to it. The dictyosomes, even when

aggregated into a Golgi apparatus, separate during mitosis and are segregated into the daughter cells, usually associating themselves with the two centrosomes (Fig. 16*B*). It is uncertain whether each dictyosome divides individually at mitosis, but some evidence has been brought forward to support this contention. In animal cells the cytoplasm as a whole divides by a process of constriction. In this process the cell elongates in the direction of the spindle during the anaphase and telophase. Following the reconstruction of the daughter nuclei in the telophase, a furrow appears at the periphery of the cell around the equatorial belt at right angles to the axis of elongation. This furrow advances toward the center of the cell until the latter is completely divided.

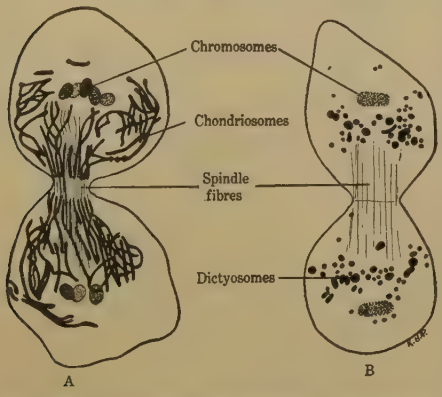

FIG. 16. — The chondriosomes and dictyosomes in mitosis. A, chondriokinesis. B, dictyokinesis. (After Bowen.)

Importance of the chromosomes. — Each daughter cell has approximately half of the cytoplasm proper, half of the chondriosomes and dictyosomes, a centrosome derived from that of the parent cell, and a nucleus built up from a set of chromosomes, each of which was produced by the division of a chromosome of the parent cell. It is apparent from the foregoing account that the key to the complexities of mitosis is the division of the chromosomes. The achromatic figure is the framework upon which this division takes place. The division of the chondriosomes and dictyosomes is still too little understood. But the chromosomes, appearing in the prophase, halved with such accuracy in metaphase and anaphase and disappearing again in the telophase, are characterized by a *constancy* in number, an *individuality* evinced in form and behavior, and a *persistence* from generation to generation. In some favorable material it has even been possible to

demonstrate that each chromosome is reconstructed in the prophase exactly as it was prior to its disappearance in the previous telophase. From the statements above, it is not unreasonable to draw the conclusion that the chromosomes are the bearers of inheritance in cell reproduction.

Meiosis. — During the two meiotic divisions by which the auxocyte gives rise to the gametes, the number of chromosomes is reduced to one-half the number characteristic of the species. Since in the ordinary somatic mitosis the number of chromosomes given to each daughter cell is exactly the same as that of the parent, it is evident that this is a peculiar type of cell division.

The heterotypic division. — It is the first of the meiotic divisions which departs most widely from the somatic type of mitosis and is, for this reason, known as the **heterotypic** division (Fig. 17).

In the prophase of this division, the chromatin gives rise to leptotene chromosomes as usual (Fig. 17*B*). Immediately following this stage the chromosomes unite in pairs to form double or **synaptene** chromosomes (Fig. 17*C*), thus apparently reducing the number of these bodies from the original **diploid** number characteristic of the species to one-half that amount, the **haploid** number. After this union, the synaptene chromosomes condense and are known as pachytene chromosomes, as in somatic mitosis (Fig. 17*D*). Then follows the diplotene stage (Fig. 17*E*) in which a longitudinal split appears. This, however, does not correspond to the plane along which the leptotene chromosomes united in synapsis, but is at right angles to this plane, thus dividing each member of the double chromosome and converting the entire body into a quadripartite chromosome known as a **tetrad.** The composition of the tetrad becomes more apparent in the concluding stage of the prophase, that of **diakinesis.** During this stage the tetrad condenses still further, thus losing its primitive shape as a long thread divided by two longitudinal splits at right angles to each other. Of these the one along which the synaptic mates joined is known as the **primary** split, and the other, by means of which each of the synaptic mates is divided, is known as the **secondary** split. The final form of the tetrad is that which it assumes upon entering the spindle (Fig. 17*F*).

The tetrad as seen in the metaphase plate is often very unlike the chromosome of the ordinary somatic mitosis. The form

varies according to the degree of condensation, the appearance of the primary and secondary splits, and the method by which the chromosomal fibers become attached. The quadripartite nature

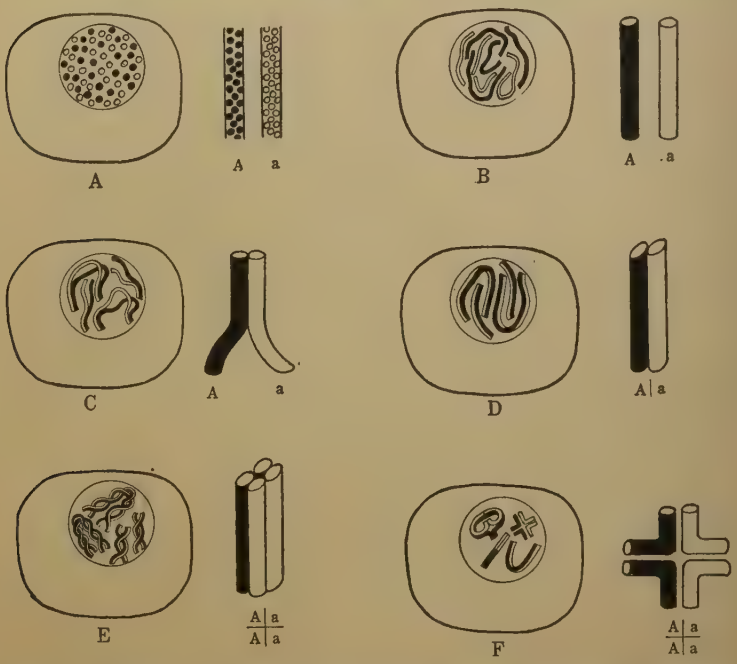

FIG. 17. — Diagram of meiosis. A, interphase, primary auxocyte. B, leptotene stage of prophase. C, synaptene stage of prophase. D, pachytene stage of prophase. E, diplotene stage of prophase, tetrads formed. F, diakinesis. To the right of each cell is shown a diagram of the behavior of one pair of chromosomes, A and a, enlarged.

of a tetrad may be expressed by the formula $\frac{\mathbf{A} : \mathbf{a}}{\mathbf{A} : \mathbf{a}}$, where **A** represents one synaptic mate and **a** the other.

In the anaphase the daughter chromosomes are bipartite, or **dyads,** and each of the separating groups contains the haploid number. The individual tetrad may separate along the primary split, thus separating $\frac{\mathbf{A}}{\mathbf{A}}$ from $\frac{\mathbf{a}}{\mathbf{a}}$ in a real **reduction division** (Fig. 18*A*), for in this case each group of chromosomes will then have

a representative of but one of each pair of synaptic mates. On the other hand, the tetrad might divide along the line of the secondary split, thus separating **A : a** from **A : a** in an **equation**

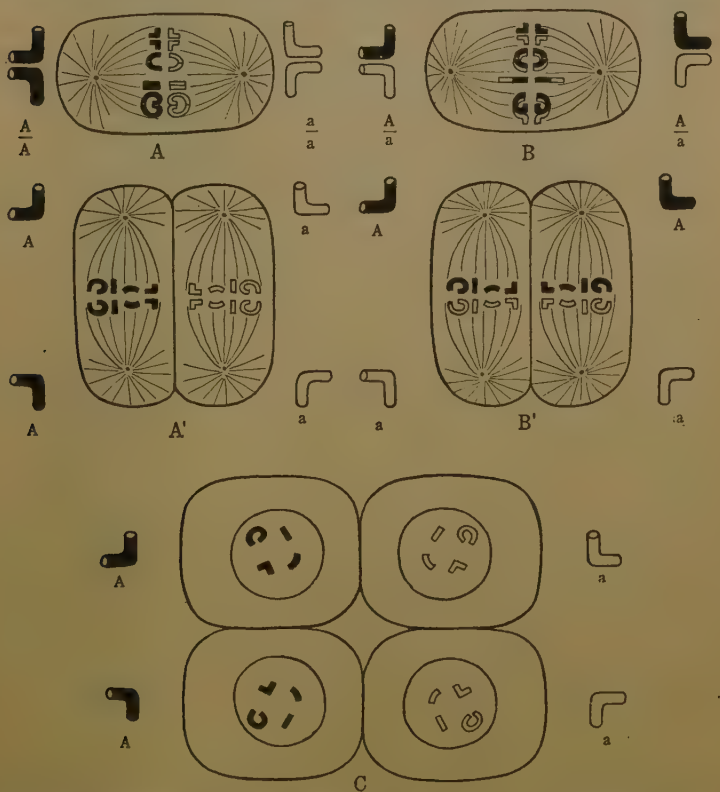

FIG. 18. — Diagram of meiosis, continued from Fig. 17. A, anaphase of first meiotic division, if reductional. A′, anaphase of second meiotic division following A. B, anaphase of first meiotic division, if equational. B′, anaphase of second meiotic division following B. C, interphase following second meiotic division after either A′ or B′, four gametes. The distribution of the tetrad $\frac{A : a}{A : a}$ is indicated as in Fig. 17.

division (Fig. 18*B*), in which each group of chromosomes has a representative of each of the original chromosomes.

In some cases the chromosomes disintegrate into their con-

stituent chromomeres in a telophase prior to the division of the auxocyte into secondary spermatocytes or oöcytes, as the case may be. In others no telophase occurs, the dyads remaining separate until the next, or **homœotypic,** division.

TABLE V

Stages of Mitosis and the Meiotic Divisions

Mitotic Stages	Cells	First Meiotic (Heterotypic) Division	Chromosomes	
			Type	Number
Prophase	One	Prophase		
Leptotene		Leptotene	Monads	Diploid
		Synaptene	Dyads	Haploid
Pachytene		Pachytene	Dyads	Haploid
Diplotene		Diplotene	Tetrads	Haploid
		Diakinesis	Tetrads	Haploid
Metaphase		Metaphase	Tetrads	Haploid
Anaphase		Anaphase	Dyads	Haploid
Telophase		(Telophase sometimes omitted)		
Interphase	Two	(Interphase sometimes omitted)		
		Second Meiotic (Homœotypic) Division		
Prophase		(Prophase sometimes omitted)		
Leptotene				
Pachytene				
Diplotene				
Metaphase		Metaphase	Dyads	Haploid
Anaphase		Anaphase	Monads	Haploid
Telophase		Telophase	Monads	Haploid
Interphase	Four	Interphase	Monads	Haploid

The homœotypic division. — In case the heterotypic division terminated in a telophase, the concluding meiotic division manifests a prophase. If the telophase was omitted, however, the dyads produced by the anaphase of the heterotypic division pass

directly upon the spindle of the homœotypic division, which is then in the metaphase stage.

In either case the anaphase of the second division results in the separation of the dyads into their component halves, or **monads.** If the first division of a tetrad was an equation division, the second must be a reduction division and *vice versa.* The end result of the two divisions is that each gamete will have one of the four parts of each tetrad, **A, A, a,** or **a.** In other words, the spermatid or oötid has a representative of only one of each pair of synaptic mates, **A** or **a** (Fig. 18*C*).

This applies, of course, to each and every pair of chromosomes in the original auxocyte. If, for example, there were eight chromosomes, **A, a, B, b, C, c, D,** and **d** in a spermatocyte, these would unite in synapsis to form four double chromosomes, **Aa, Bb, Cc,** and **Dd.** From these would be formed at the diplotene stage four tetrads, $\frac{A:a}{A:a'}\ \frac{B:b}{B:b'}\ \frac{C:c}{C:c}$ and $\frac{D:d}{D:d}$. Following the two meiotic divisions, equation and reduction, regardless of their order, the final spermatids would have four chromosomes (the haploid number of monads), but only one representative of each synaptic pair. The possible combinations are 2^4 or 16, namely, **ABCD, ABCd, ABcd, Abcd, ABcD, AbcD, AbCD, AbCd, aBCD, aBCd, aBcd, abcd, aBcD, abcD, abCD, and abCd.** Accordingly, the number of different types of gametes which may be formed can be determined from the formula 2^n when n is the haploid number of chromosomes characteristic of the species.

Fertilization. — It now becomes apparent that in the union of the pronuclei during fertilization, each pronucleus contributes a haploid number of chromosomes, so that the fertilized egg has the diploid number. If the number of chromosomes were not halved in meiosis, it would be doubled in each generation at the time of fertilization. The fact that each gamete contributes a set of chromosomes explains the existence of the pairs of chromosomes that unite during the meiosis in which the gametes for the next generation are formed. Each synaptic pair consists of one chromosome from the male pronucleus and one from the female pronucleus. During meiosis either the paternal or maternal chromosome is selected, and in this way the different types of gametes are constituted. At the next fertilization an entirely

different type of zygote is formed by the union of unlike gametes. Thus, in the example in the preceding paragraph of an animal with a diploid number of 8 chromosomes, 16 different types of gametes might be formed (Fig. 19). From these there is the possibility of 16^2, or 256, different combinations in fertilization. Furthermore, it should be noted that during synapsis there frequently takes place an orderly interchange of material between the synaptic mates, by means of which the number of recombinations of chromatic material is greatly increased (see page 55).

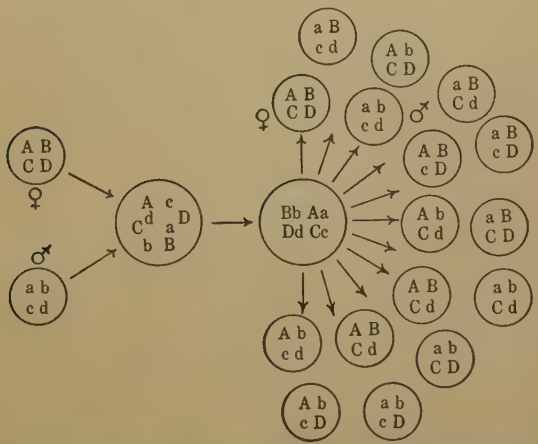

FIG. 19. — Diagram showing the distribution of the chromosomes in fertilization and meiosis. (After Wilson, "The Cell in Development and Heredity," copyright, 1925, by the Macmillan Co.)

Sex-chromosomes. — The phenomena of meiosis and fertilization are further elucidated by the behavior of certain chromosomes which seem to be connected with the determination of sex and are, therefore, known as **sex-chromosomes.**

Male digamety, X-O type. — In many animals, such as the insect Protenor, the male has one chromosome less than the female, the numbers in Protenor being 13 and 14, respectively (Fig. 20). If the synaptic pairs are assembled, it is clear that the male has 6 pairs of ordinary chromosomes, or **autosomes,** and an extra **heterosome** or **X-chromosome;** while the female has 7 pairs, of which 6 pairs are autosomes and the seventh pair

is made up of heterosomes or **X**-chromosomes. The autosomes may be disregarded. In the female the **X**-chromosomes unite

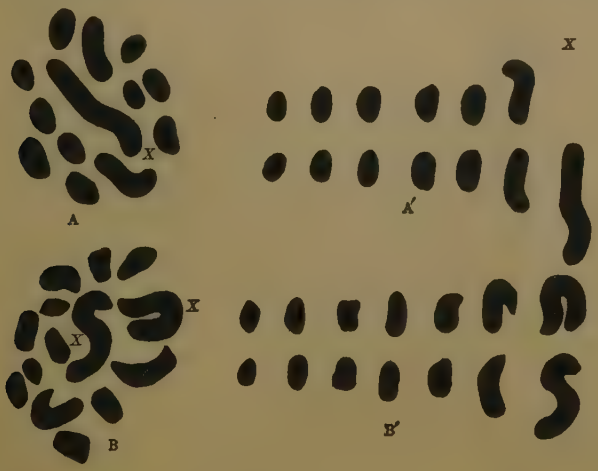

Fig. 20. — Chromosomes of Protenor. A, male diploid group. B, female diploid group. A′, B′, the same arranged in pairs. (After Wilson, "The Cell in Development and Heredity," copyright, 1925, by the Macmillan Co.)

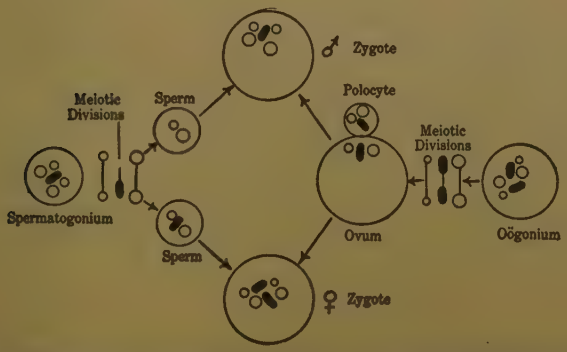

Fig. 21. — Diagram of male digamety, X–O type. Spermatogenesis to left, oögenesis to right. (After Wilson.)

in synapsis, form a tetrad, and are segregated in the meiotic divisions so that every oötid has one **X**-chromosome. In the male, on the other hand, the single **X**-chromosome has no synap-

tic mate and so goes on the spindle of the first meiotic division as a dyad, which is carried to one pole of the spindle entire. In the second meiotic division the dyad is divided as usual. The end result is that only half the spermatids receive an **X**-chromosome, and two classes of spermatozoa are formed according to the presence or absence of an **X**-chromosome. If in the succeeding fertilization a sperm with an **X**-chromosome unites with the egg, the female combination (**XX**) is restored; while if a sperm without the **X**-chromosome unites with the egg, the male combination (**XO**) is formed (Fig. 21). These facts have led to the chromosomal theory of the determination of sex, in addition to supporting the evidence for meiosis and fertilization.

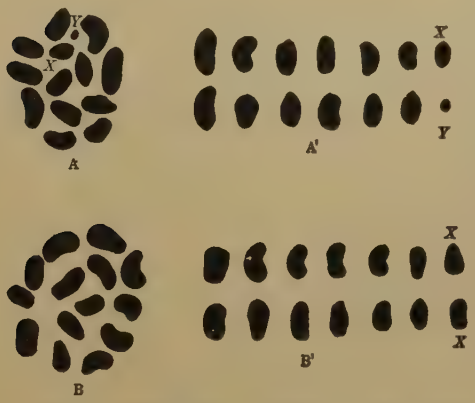

FIG. 22. — Chromosomes of Lygæus. A, male diploid group. B, female diploid group. A′, B′, the same arranged in pairs. (After Wilson, "The Cell in Development and Heredity," copyright, 1925, by the Macmillan Co.)

Male digamety, X-Y type.—The sexes do not differ in chromosome number in every instance, however, for in many animals, like the insect Lygæus (Fig. 22), the **X**-chromosome of the male is furnished with a synaptic mate which differs from it in size, form, and probably composition, and is therefore known as the **Y-chromosome.** With regard to the heterochromosomes, therefore, the female forms in meiosis the tetrad $\frac{\mathbf{X} : \mathbf{X}}{\mathbf{X} : \mathbf{X}}$, and every ovum has an **X**-chromosome. The male forms a tetrad $\frac{\mathbf{X} : \mathbf{Y}}{\mathbf{X} : \mathbf{Y}}$, and the spermatozoa therefore have an **X**- or a **Y**-chromosome. Fertilization by a sperm bearing the **X**-chromosome results in the development of a female (**XX**), while if a sperm bearing a **Y**-chromosome enters the egg the embryo will give rise to a male (**XY**).

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Female digamety. — As an exception to the general rule among the vertebrates, the birds, and some fish, are characterized by the occurrence of sexual dimorphism in the egg, that is, by female digamety. The cytological details are difficult to interpret, but the theoretical explanation is that the female has two dissimilar sex-chromosomes known as **W** and **Z**, while the male possesses two similar sex-chromosomes of the **Z** type. In the meiosis of the oöcyte, therefore, a tetrad $\frac{W : Z}{W : Z}$ is formed, and the ovum receives either a **W**- or a **Z**-chromosome. The spermatocyte forms a tetrad $\frac{Z : Z}{Z : Z}$, and all sperm carry one **Z**-chromosome. In this group, therefore, it is the ovum which determines the sex of the embryo rather than the sperm. This explanation agrees with the data obtained from genetics.

Mendelian inheritance. — It has already been said that the behavior of the chromosomes strongly suggests the probability that they are the material carriers of hereditary units. We shall now examine a few cases in which the behavior of the chromosomes in meiosis and fertilization parallels the behavior of the hereditary units (**genes**) in the transmission of variable characters from parent to offspring.

One pair of characters. — This type of inheritance was discovered by Mendel (1865) when he crossed peas that bore a yellow seed with those producing a green seed. Seeds from this cross were all yellow, showing that the gene for yellow seed color is **dominant** to the gene for green, while the latter gene is **recessive** to the former. When the hybrid plants were self-fertilized it was found that three-quarters of the seeds were yellow, while the remainder were green. This is the famous **Mendelian ratio** of 3 : 1. On self-fertilizing the gametes of this hybrid generation, one-third of the plants from yellow seeds produced yellow seeds; the remaining two-thirds again produced the Mendelian ratio, 3 yellow to 1 green; while the plants from green seeds produced only green seeds. In other words, the true ratio in the second generation is 1 pure yellow; 2 hybrid yellow; 1 pure green.

Let us now suppose that the gene for yellow is located in one member of a synaptic pair of autosomes, while the alternative

(**allelomorphic**) gene for green is located at a corresponding point in the other member of the same pair, and call these chromosomes **y** and **g**, respectively. In the fertilization by which the hybrid first generation was produced, one parent contributed a **y** chromosome, the other a **g** chromosome. In the meiosis following, **y** and **g** conjugate in synapsis, form a tetrad, $\frac{y : g}{y : g}$, and are distributed to the gametes so that half of these have a **y** chromosome and half a **g** chromosome. In the zygotes of the second generation there are the following possibilities: 1 **yy** combination, 2 **yg** combinations, and 1 **gg** combination, which is exactly the Mendelian ratio stated above (Fig. 23).

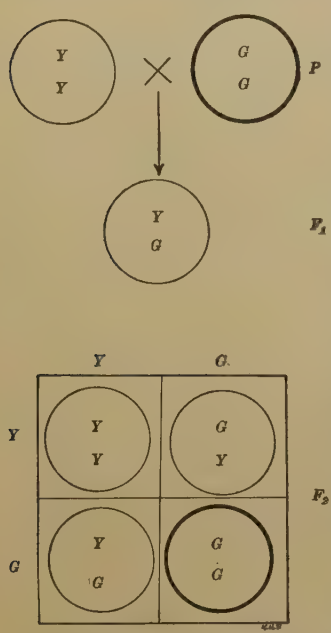


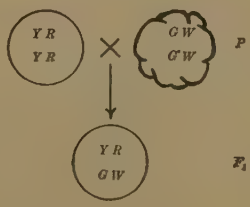
FIG. 23. — Diagram of Mendelian inheritance involving one pair of contrasting characters, yellow and green. The circles represent zygotes, the letters outside the checkerboard represent the gametes of the second generation. P, parental generation. F_1, first filial generation. F_2, second filial generation. (After Woodruff, "Foundations of Biology," copyright, 1922, by the Macmillan Co., modified.)

Two or more pairs of characters. — When the inheritance of two characters which segregate freely is studied, as in the classical case of Mendel in which he crossed peas from yellow round seeds with peas from green and wrinkled seeds, the parallel again holds true. Let us assume that the genes for the characters round and wrinkled are located in a pair of synaptic autosomes other than those in which the genes for the characters yellow and green are borne. The hybrid seeds were yellow and round, showing that round is dominant to wrinkled.

The hybrid must have the chromosomes **y**, **g**, **r** and **w**; **y** and **r** from one parent, **g** and **w** from the other.

Two tetrads are formed from these chromosomes in meiosis,

$\frac{y : g}{y . : g}$ and $\frac{r : w}{r : w'}$, and the possible gametic combinations are four in number, **yr, yw, gr** and **gw**. In the zygotes formed by these there are 16 possible recombinations (4^2); and of these, owing to the dominance of yellow and round, there will be 9 of the yellow round type, 3 yellow wrinkled, 3 green round, and 1 green wrinkled. This is the same ratio obtained by Mendel (Fig. 24). Exactly similar results would be obtained in the second combination if a yellow wrinkled plant were crossed with one of the green round type, showing that the characters segregate freely.



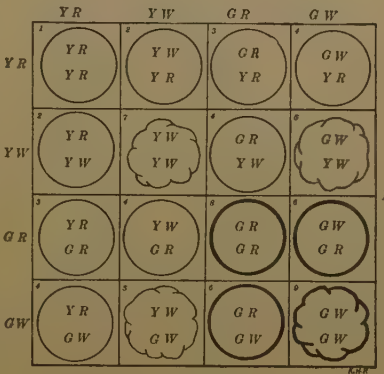


FIG. 24. — Diagram of Mendelian inheritance, involving two pairs of contrasting characters not located in the same chromosomes. Same conventions as in Fig. 23. (After Woodruff, "Foundations of Biology," copyright, 1922, by the Macmillan Co., modified.)

Two pairs of linked characters. — The characters with which Mendel worked segregated freely, showing that their genes were not borne in the same chromosome. Later studies have shown that some characters do not segregate, and this leads to the assumption that their genes are carried in the same chromosome and, therefore, are inherited together. Let us suppose that, in the fruit-fly, Drosophila, the genes for the characters grey body and long wing are carried in one member of a synaptic pair of autosomes, and that those of the allelomorphic characters, black body and vestigial wing, are carried in the synaptic mate. We should expect that the hybrid would show the dominant characters grey and long, and that in the second generation all

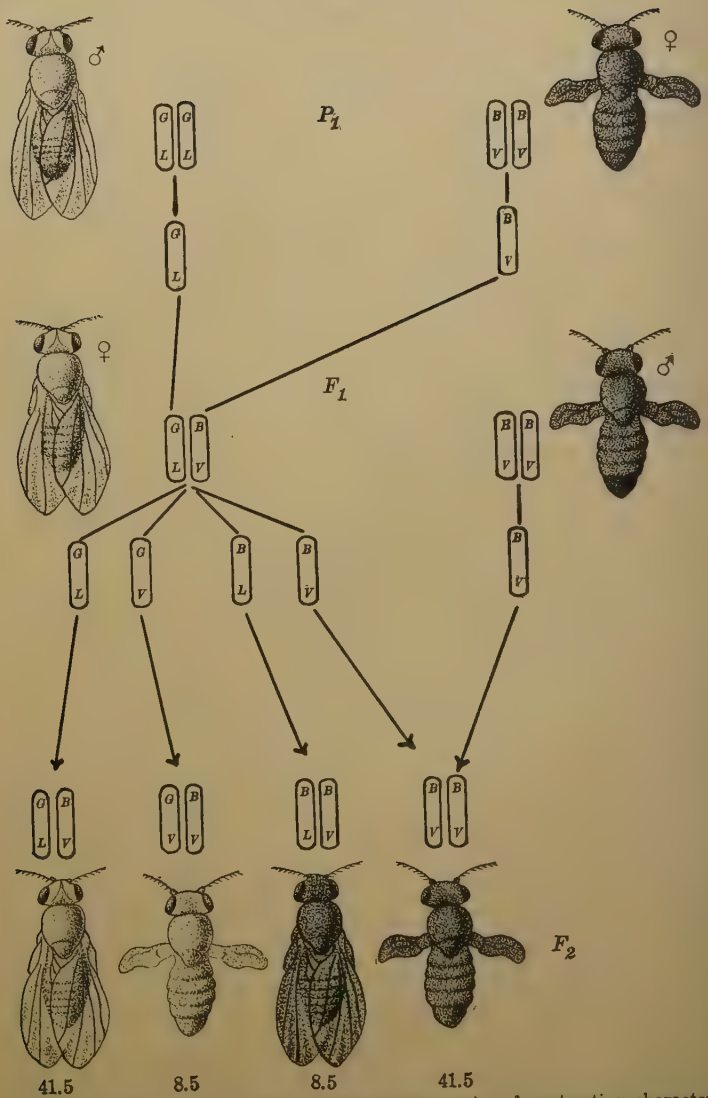


FIG. 25. — Diagram of inheritance involving two pairs of contrasting characters, grey body color and black, long wings and vestigial. The genes for grey or black and long or vestigial are in the same chromosome. The hybrid F_1 female is back crossed to a black vestigial male. The two cross over classes, grey vestigial and black long, make up 17% of the back-cross generation (here marked F_2). (Modified from a figure of Morgan.)

flies with grey body color would have long wings and, conversely, that all those with black body color would have vestigial wings. On the contrary actual experiments result in the production of a few F_2 flies with grey body color and vestigial wings and with black body color and long wings (cross-over types). In order to simplify the experiment, a hybrid female (F_1) is back-crossed to a double recessive male (Fig. 25). All the gametes produced by the male are the same (BV), so that the different classes of flies in the F_2 generation are due to the gametic types produced by the female. Seventeen per cent of the flies in this generation are

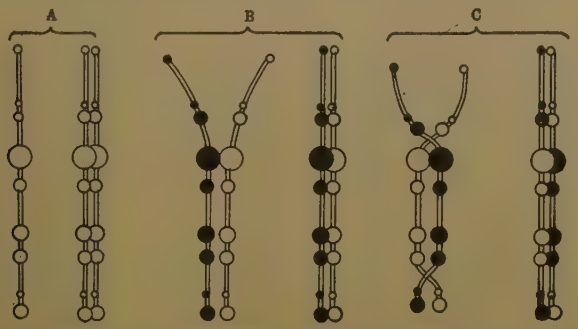


FIG. 26. — Diagram comparing mitosis, synapsis, and crossing over. A, longitudinal division of a chromosome in mitosis. B, side by side union of chromosomes in synapsis without crossing over. C, synapsis with two cross-overs. (After Wilson, "The Cell in Development and Heredity," copyright, 1925, by the Macmillan Co., modified.)

cross-overs, showing that an interchange of genes has taken place during the maturation of the eggs. This interchange of genes must take place during synapsis (Fig. 26*C*), and it is a reasonable assumption that the farther apart the genes are located in the chromosomes, the greater must be the possibility of interchange. Acting on this hypothesis, Morgan and his associates have mapped out the position of several hundred genes in the four pairs of chromosomes of Drosophila.

Sex-linked inheritance. — Perhaps the most convincing evidence of the parallelism between the behavior of the chromosomes and that of the genes is that offered by the inheritance of characters associated with sex determination. A good example is

that shown in the inheritance of Daltonian color-blindness in man, the inability to distinguish red from green. If this character manifests itself in a woman, whose mate has normal vision, it will be shown also in her sons but not in her daughters, a phenomenon known as criss-cross inheritance. We will make the primary assumption that the gene for this sex-linked character is borne in the sex-chromosome, and let **X** represent a sex-chromosome that carries the gene for the allelomorphic char-

FIG. 27. — Diagram of sex-linked inheritance. A Daltonian color blind female, represented in black, transmits to sons. The X-chromosome with the gene for color blindness indicated by X. (After Morgan.)

acter, normal vision, while **x** may represent a sex-chromosome carrying the gene for Daltonian color-blindness, and **Y** stand for the **Y**-chromosome. The formula for a color-blind man will then be **xY**; for one with normal vision, **XY**; for a color-blind woman, **xx**; for one with normal vision, **XX** or **Xx**, since this character is recessive.

If a color-blind woman (**xx**) marries a man with normal vision (**XY**), her sons will be color-blind (**xY**) since the sex-chromosome must come from the mother. Her daughters, on the other hand, will appear normal in this respect, although they will all carry the recessive gene received from the mother (**xX**). This statement is proved by the fact that if the daughter of a color-blind mother marries a man with normal vision, half of her sons will be color-blind (**xY**) while half will be normal (**XY**), according to

which of the sex-chromosomes they inherited. Half of her daughters will carry the recessive gene (**xX**), half will not (**XX**), but all will have normal vision themselves (Fig. 27, 27*a*).

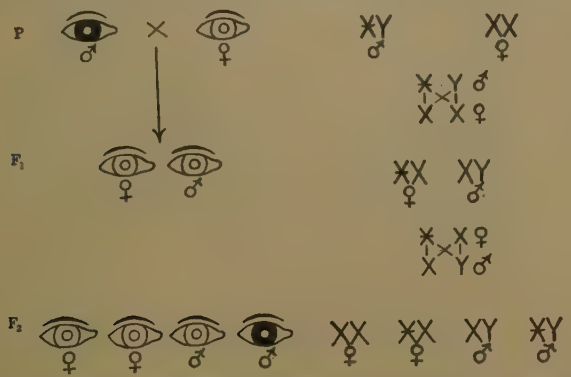


Fig. 27*a*. — Diagram of sex-linked inheritance from the male. Compare Fig. 27. (After Morgan.)

The frog. — The diploid number of chromosomes in the frog is 26, and the sex-chromosomes of the male are of the **X-Y** type (Fig. 28*A*).

The chick. — The diploid number of chromosomes is 35 or 36 (Fig. 28*B*). The sex-chromosomes have not been positively

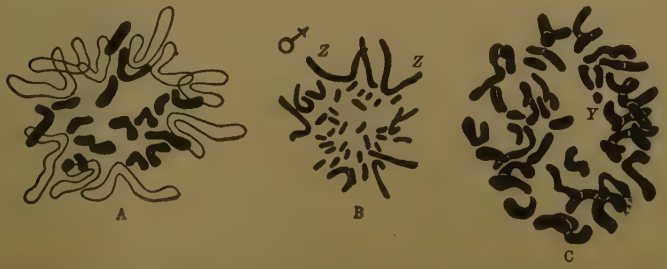


Fig. 28. — Metaphase plates of male diploid chromosome groups. A, frog (after Witschi). B, chick (after Hance). C, man (after Painter).

identified but genetic evidence indicates that the sex-chromosomes of the female are of the **O-Z** or the **W-Z** type.

Man. — The diploid number of chromosomes in man, according to the most recent researches, is 48. The sex-chromosomes are

of the **X-Y** type (Fig. 28*C*). It is interesting to note that with 48 chromosomes the possible types of gametes number 2^{24}, or 16,777,300, and that, from these, 281,475,000,000,000 types of zygotes are possible. Over 60 Mendelian characters are known; of these, 5 are sex-linked.

Cytoplasmic inheritance. — Although the behavior of the chromosomes in meiosis and fertilization supplies such a far-reaching explanation of the mechanism involved in heredity, the objection has often been made that the Mendelian characters, to which the chromosome theory applies directly, are important in determining the individual characters rather than those of the race. Accordingly, attempts have been made to discover in the cytoplasm, as well as in the nucleus, self-perpetuating units which might transmit racial characters. In the next chapter (page 67), it is pointed out that some eggs betray a regional differentiation, usually by the stratification of the egg into different layers, which are known as organ-forming substances (Figs. 29, 58). If these substances be displaced during subsequent development, the organs normally arising from them are similarly displaced or develop abnormally. Assuming that the ovum represents an organization of such substances prior to fertilization, Loeb spoke of the cytoplasm as "the embryo in the rough," to which the nucleus, after fertilization, adds only the finer details. Some have attempted to identify these cytoplasmic determinants as the chondriosomes, dictyosomes, and other formed bodies. The transmission of hereditary characters by means of cytoplasmic determinants is still unproven.

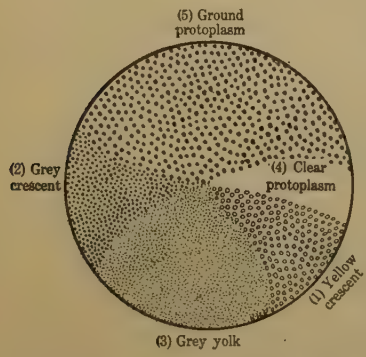


Fig. 29. — Side view of egg of Styela just preceding first cleavage. (After Conklin.)

Summary. — **The gametes are atypical cells which differ both from each other and from the generalized type of cell. The egg resembles most closely the generalized type, from which it differs in the absence of a centrosome. It is large, quiescent, and protected by envelopes.**

The sperm is almost devoid of cytoplasm, and is small, active, and naked.

The gametes are derived from primordial germ cells which produce gonia in the developing ovary or testis. These gonia become auxocytes, large germ cells about to undergo two meiotic divisions. The spermatocytes produce four spermatids, each of which becomes a spermatozoön. The oöcytes produce four oötids, of which one retains the greater part of the cytoplasm of the parent cell and is the ovum; the others, minute polocytes, disintegrate. When egg and sperm are brought together, the sperm are attracted to the egg, and one penetrates its cell membrane by mutual interaction, whereupon the nuclei of the egg and sperm conjugate.

The two meiotic divisions are mitotic; but the first, or heterotypic, division is unique in that following the leptotene stage of the prophase the chromosomes unite in pairs, by a process known as synapsis. As a result, the chromosomes of the metaphase are halved in number but quadripartite in composition (tetrads). Through the distribution of the four parts of each tetrad through the four spermatids or oötids in the two meiotic divisions, the gametes differ among themselves with respect to the presence of either member of any pair of chromosomes. In the zygote after fertilization, the number of chromosomes is doubled, one member of each pair coming from either parent. The problem of sex determination is explained by the behavior of the so-called sex-chromosomes. The behavior of the chromosomes in meiosis and fertilization offers an explanation of Mendelian inheritance, linkage, and sex-linked inheritance. It has been suggested that certain organ-forming substances in the cytoplasm of the egg may be responsible for some of the phenomena of inheritance.

REFERENCES

Bowen, R. H. 1924. On the Acrosome of the Animal Sperm. Anatomical Record, XXVIII, 1.

Brachet, A. 1921. Traité d'Embryologie, Book 1.

Cowdry, E. G. 1924. General Cytology.

Graham-Kerr, J. 1919. Textbook of Embryology, Vol. II, Chap. 1.

Hertwig, O. 1906. Handbuch, Vol. 1, Chaps. 1 and 2.

Jenkinson, J. W. 1913. Vertebrate Embryology, Chaps. 3 and 4.

Kellicott, W. E. 1913. General Embryology, Chaps. 2, 3, 4 and 5.

Korschelt and Heider. 1902. Lehrbuch, Chaps, 4, 5 and 6.

Lillie, F. R. 1919. Problems of Fertilization.

Loeb, J. 1916. The Organism as a Whole from a Physicochemical Viewpoint.

Morgan, T. H., and others. Rev. ed. 1923. The Mechanism of Mendelian Inheritance.

Wilson, E. B. 1925. The Cell, Chaps. 1–6, 9–12.

CHAPTER III

THE GERM LAYERS

The zygote is a complete and balanced cell, with a cell body which is principally maternal in origin, and a nucleus to which each gamete has contributed one haploid set of chromosomes, bearing the genes for the innumerable characteristics of the developing embryo. During the period which follows, these characteristics appear in an orderly series from simpler structures to those that are more complex.

A. THE FORMATION OF THE BLASTULA

The first activity manifested by the developing zygote is the renewal of the mitotic divisions terminated by the two meiotic divisions of gametogenesis. This first division of the zygote is a normal mitosis, with the single peculiarity that each of the conjugate pronuclei undergoes a separate karyokinetic prophase. Indeed, in some eggs the paternal and maternal groups of chromosomes may be distinguished upon the spindle in the metaphase of this mitosis, and, sometimes, in many successive divisions (Fig. 30). This is especially true of hybrids in which the ovum of one species has been fertilized by the sperm of another. Usually, however, the paternal and maternal chromosomes intermingle in the telophase.

FIG. 30. — Chromosomes in anaphase of first cleavage of a hybrid between female Menidia and a male Fundulus. (After Moenkhaus.)

The ovum, it will be remembered, is an excessively large cell, and during this period of cleavage no growth is apparent. Consequently, the products of these cell divisions, the blastomeres, become smaller with each division. At the end of this period of the life history the developing embryo is called a **blastula.**

The cleavage pattern. — Certain rules have been formulated to express the simpler geometrical relationships of the blastomeres. The first are those of Sachs: (1) cells typically tend to divide into equal parts; (2) each new plane of division tends to intersect the preceding one at right angles. Sachs' rules are supplemented by those of Hertwig: (1) the typical position of the nucleus (and hence of the mitotic figure) tends towards the center of its sphere of influence, i.e., of the protoplasmic mass in which it lies; (2) the axis of the spindle typically lies in the longest axis of the protoplasmic mass, and division therefore tends to cut this axis transversely. The rate of division is governed by the rule of Balfour: the rate of cleavage is inversely proportional to the amount of yolk present.

Three types of cleavage pattern are distinguished in vertebrate embryology. **Holoblastic** cleavage (Fig. 31*A*) is characteristic

FIG. 31. — Diagram of cleavage types. A, holoblastic. B, intermediate. C, meroblastic.

of oligolecithal eggs. It is complete, equal, and regular; that is, at each cleavage the cell is completely divided into two equal parts and the number of cells increases in a geometrical sequence, 2, 4, 8, 16, 32 . . . Lecithal eggs, like those of the frog, have an **intermediate** (Fig. 31*B*) type of cleavage, which is complete, unequal, and irregular. The cells are completely divided but into unequal portions, so that the cells at the apical pole are the smaller, and the number of cells increases more rapidly at the apical pole, the sequence being 2, 4, 8, 12, 16, 24, 32 . . . In macrolecithal eggs, like those of the fowl, cleavage is **meroblastic** (Fig. 31*C*); it is incomplete, unequal, and irregular, for the cleavages do not pass through the yolk, and they leave a cap of unequal and irregularly increasing cells on an undivided mass of yolk.

Amphioxus. — In the egg of Amphioxus (Fig. 32), which is oligolecithal, cleavage is holoblastic. The first cleavage commences as a depression at the apical pole; it later assumes a groove-like form and elongates until it becomes a wide **meridional** furrow extending around the egg. This constriction deepens until the two hemispheres are completely divided, when each blastomere rounds up into a spherical shape. The second cleav-

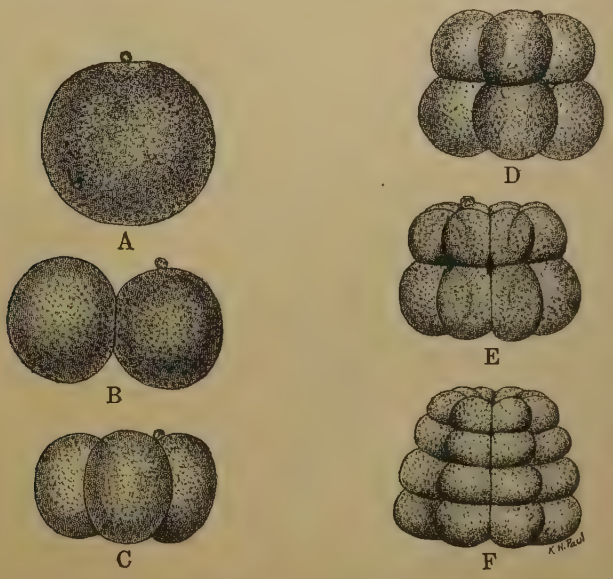


FIG. 32. — Cleavage in Amphioxus. A, before cleavage. B, first cleavage. C, second cleavage. D, third cleavage. E, fourth cleavage. F, fifth cleavage. (After Hatschek.)

age also commences at the apical pole and is meridional, but it is at right angles to the first, following the second rule of Sachs. The third plane of cleavage is at right angles to both the first and second, and hence would be **equatorial** if the egg were completely isolecithal. But as the yolk is concentrated at the abapical pole, the nucleus, following Hertwig's first rule, is in the center of the protoplasm, i.e., in the egg axis slightly nearer the apical pole. So the third cleavage plane is nearer the apical pole, and accordingly is called **latitudinal.** The quartette of cells

in the apical hemisphere, being smaller than those of the abapical, are called **micromeres,** while the latter are known as **macromeres.** The fourth division divides each of the eight existing blastomeres in two. There are two planes of cleavage, each meridional, at right angles to the third, and also at right angles to each other. In the fifth cleavage, thirty-two cells are produced, again by two planes of cleavage, at right angles to the planes of the fourth, but this time latitudinal and parallel to each other. From this time on cleavage becomes more and more irregular. The early cleavages have been fairly regular; each has divided the entire egg mass, and the blastomeres, with the exceptions noted, have been almost equal. The blastomeres round up as each cleavage is completed, and in this way a small cavity is left between them. This is called the **segmentation cavity,** because of the way in which it is formed.

The frog. — As an example of the lecithal type with intermediate cleavage, the frog's egg (Fig. 33) is chosen. Here the

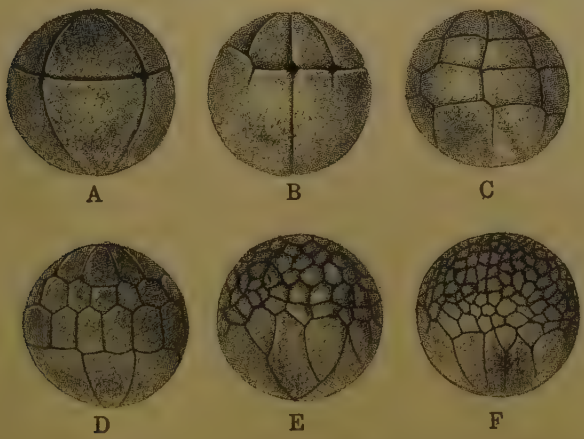


FIG. 33. — Cleavage in the frog's egg. A, third cleavage. B, fourth cleavage (12 cells). C, fifth cleavage. D, sixth cleavage. E, F, later stages. (After Morgan.)

first division commences as a depression at the apical pole; it elongates and extends around the egg as a shallow furrow until the ends meet at the abapical pole. The constriction extends inward and eventually bisects the egg into two blastomeres which

round up very slightly. The second division also has a meridional plane, through the apical pole but at right angles to the first. The rounding up of the blastomeres produces a **polar furrow.** The third division is latitudinal, about 20° above the equator, and the micromeres are considerably smaller than the macromeres. A segmentation cavity is now established. Theoretically, the fourth and fifth planes of cleavage bear the same

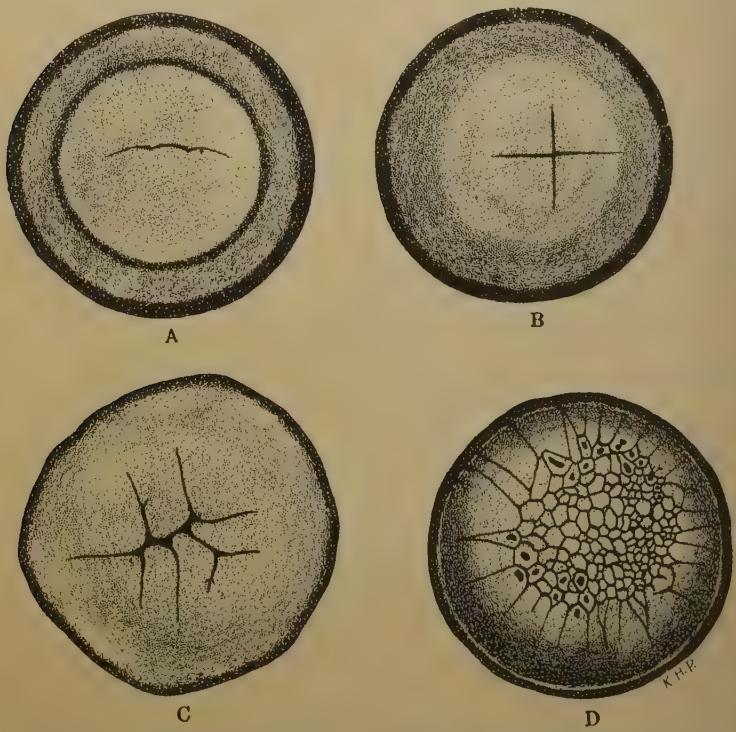


FIG. 34. — Cleavage in the hen's egg. A, first cleavage. B, second cleavage. C, third cleavage. D, later cleavage. (A, B, D, after Kölliker. C, after Patterson.)

relationships to the earlier ones as do those of Amphioxus, but they actually become considerably more irregular. The two planes of the fourth cleavage often fail to pass through the abapical pole and hence become vertical rather than true merid-

ional planes. As these planes originate in the apical hemisphere, the micromeres are divided before the macromeres, so that a twelve-cell stage intervenes between the eight-cell and sixteen-cell stages. Similarly, following Balfour's rule, the latitudinal cleavage plane in the apical hemisphere of the fifth division appears first, so that there is a twenty-four-cell stage before the appearance of the plane in the abapical hemisphere brings about the thirty-two-cell stage.

The chick. — In macrolecithal eggs with meroblastic cleavage, such as that of the fowl, only the protoplasm of the egg, i.e., the **blastodisc,** is divided, and the cleavage planes do not segment the yolk (Fig. 34). The first furrow commences at the apical pole and extends outward toward the edges of the blastodisc. The second is formed by two furrows, at right angles to the first, one in each blastomere, which grow toward the first furrow and also toward the edge of the blastodisc. They may join the first furrow at approximately the same point or at separate points, in which case a polar furrow is formed. These

FIG. 35. — Cleavage in the bat's egg. A, first cleavage. B, second cleavage. C, third cleavage. D, later stage. (After Van Beneden.)

four cells are incomplete, as the furrows do not extend all the way to the yolk nor to the edge of the blastodisc, but remain connected both below and at their margins. From this point on, cleavage is irregular. Some cleavage planes are circular and cut off central cells from marginal. These may be compared with the latitudinal planes of the holoblastic type. Others are radial, like the first and second. Still others are **tangential** and divide the central cells into upper and lower layers, as in the frog's egg. No segmentation cavity appears.

Man. — The cleavage of the human embryo has not been worked out, but in all the placental mammals studied (Fig. 35)

the type of cleavage is holoblastic, as would be expected in an oligolecithal type of egg.

Cleavage and the sperm. — From this account it will be seen that, given the plane of the first cleavage, the planes of division immediately succeeding can be predicted approximately in accordance with the laws stated at the beginning of this chapter. In the case of the frog's egg, the first cleavage plane usually bisects the grey crescent, which, as has been seen, is formed approximately opposite the point of entrance of the spermatozoön. If this generalization is correct, the first plane of cleavage is determined by two points, the apical pole and the point of entrance of the spermatozoön. In other forms, such as the toad-fish, the direction of the first plane of cleavage is quite variable.

Localization. — Cleavage is something more than a process of multiplication by which the unicellular egg becomes a multicellular blastula. The blastomeres soon manifest differences in form and behavior, and especially in regard to the structures to which they will give rise. This generalization is based on two lines of evidence: one, the study of the history of the individual cells in normal development, known as **cell-lineage**; the other, the study of the history of individual blastomeres experimentally separated from the dividing egg.

Cell-lineage. — In Amphioxus and the frog, for example, the first cleavage usually divides the egg sagittally into two blastomeres, from one of which the right half of the embryo is formed, while the other gives rise to the left side. The region of the so-called grey crescent in the frog's egg, is divided bilaterally in about 75 per cent of all cases. However, variations of as much as 90° have been observed without any disturbance in later development. Many investigators have shown that modification of the position or the order of succession of the cleavage planes may be induced experimentally without harmful effects on later development. In one of the protochordate ascidians, *Styela partita*, it has been observed that any alteration of the cleavage planes results in profound disturbances in the course of development. Accordingly, two classes of cleavage have been recognized: **determinate,** in which the pattern of cleavage determines the differentiation of the parts of the em-

bryo, as in Styela; and **indeterminate,** in which the pattern of cleavage can be altered without affecting the course of development, as in the frog.

Isolated blastomeres. — Similar results were obtained from the study of separated blastomeres (Fig. 36). It was found that in some animals, such as Amphioxus, isolated blastomeres in the two-cell, four-cell and, less frequently, eight-cell stage, would continue development, giving rise to dwarf blastulæ. The isolated blastomeres in the two-cell stage indeed produce dwarf larvæ. On the other hand, the isolated blastomeres of Styela produce only the structures to which they would give rise as part of the egg as a whole.

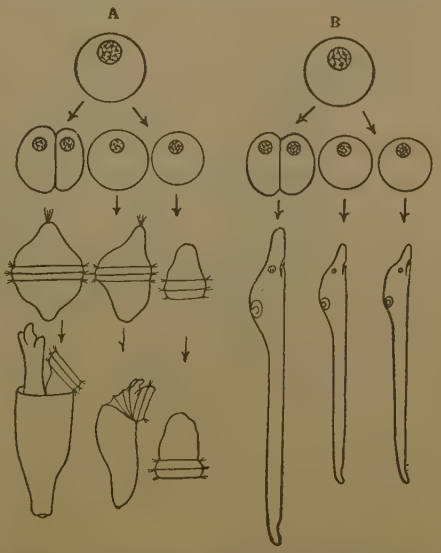


FIG. 36. — Diagram to show fate of isolated blastomeres in determinate and indeterminate cleavage. A, determinate cleavage in Dentalium; at left, complete embryo from normal egg; at right, partial embryos from each of first two blastomeres, artificially separated. B, indeterminate cleavage in Amphioxus, same arrangement. (After Wilson.)

Organ-forming substances. — The reconciliation of these discordant results is based on the existence of the organ-forming substances alluded to in the last chapter. In Styela, for example, some five different substances have been identified: (1) a yellow crescent, which produces the larval mesoderm; (2) a light-grey substance, derived from the grey crescent, which forms the notochord and neural plate; (3) a grey yolk, from which the endoderm is derived; and (4) a transparent, clear protoplasm, which gives rise to the ectoderm (Fig. 29). These separate organ-forming substances are distributed to the different blastomeres in the cleavage divisions, which may be either determinate or indeterminate.

Thus in Styela the first cleavage divides the yellow crescent equally between the first two blastomeres; this is an indeterminate division. In the second cleavage, this material passes over into the two posterior blastomeres, while the anterior pair is entirely devoid of this substance; this is a determinate division. From this it is evident that in an indeterminate egg the first cleavage

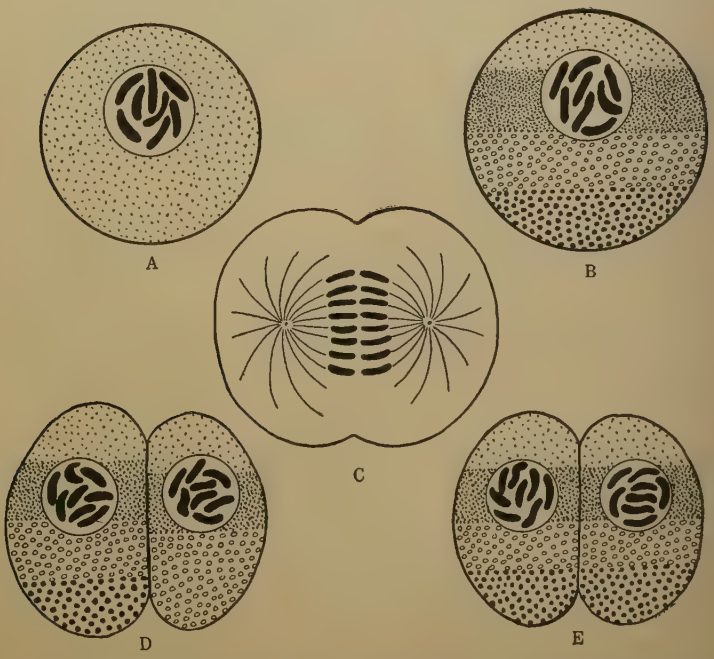


Fig. 37. — Diagram to show division of organ-forming substances. A, immature egg. B, mature egg with cytoplasmic zones. C, first cleavage. D, determinate division. E, indeterminate division. (After Wilson, modified.)

must be indeterminate in respect to all organ-forming substances, while in a determinate egg, the first cleavage must be determinate in respect to at least one organ-forming stuff (Fig. 37). At the end of the cleavage period, therefore, the blastomeres must differ potentially even though no apparent differences are to be seen in the cells of the blastula.

The blastula. — The blastula, or end-product of the period of cleavage, is the aggregate of cells produced by the division of the zygote, before any growth has taken place, and in which the blastomeres have not rearranged themselves into the germ layers. Among the chordates the blastulæ of the different classes differ widely in appearance (Fig. 38). The typical blastula of the invertebrate animals is seen only among the

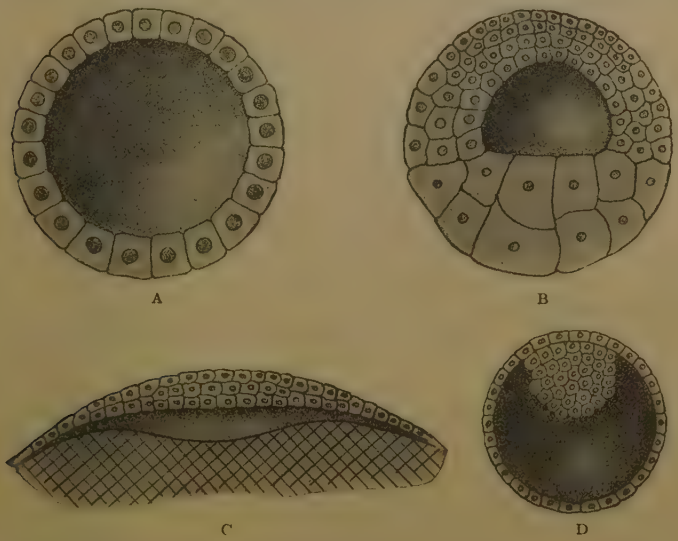


FIG. 38. — Diagrams of blastulæ. A, after holoblastic cleavage. B, after intermediate cleavage. C, after meroblastic cleavage. D, mammalian blastocyst.

protochordates. Just as in the cleavage pattern, the type of blastula is chiefly influenced by the amount of yolk present. In oligolecithal eggs the blastula (Fig. 38*A*) is composed of a single layer of blastomeres, called the **blastoderm,** surrounding a cavity derived from the segmentation cavity, called the **blastocœle,** which does not communicate with the exterior. In lecithal eggs (Fig. 38*B*), the blastocœle is eccentric or displaced toward the apical pole, and the blastoderm is several layers of cells in thickness. In macrolecithal eggs with meroblastic cleavage (Fig. 38*C*) the blastoderm is a small cap of cells surmounting the undivided yolk and separated from it by a blastocœle which differs from

that of the preceding types in that it is not derived from a segmentation cavity but is formed by the withdrawal of the blastoderm from the yolk below and originates as a yolk-cavity. The mammalian **blastocyst** (Fig. 38*D*) is an aberrant type of blastula, described below, with a yolk-cavity.

Amphioxus. — In the development of Amphioxus we find a good example of the ideal blastula (Fig. 39*a*). The blastomeres are arranged in a single layer around the enlarged segmentation cavity which is entirely cut off from the exterior and now known as the blastocœle. The blastomeres at the apical pole are

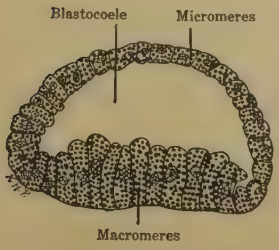


FIG. 39*a*. — Section of blastula of Amphioxus. (After MacBride.)

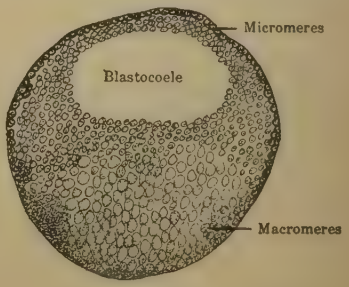


FIG. 39*b*. — Section of blastula of frog. (After Brachet.)

micromeres; those at the abapical pole are macromeres; while the cells at the equatorial belt are transitional in type.

The frog. — The blastula of the frog (Fig. 39*b*) resembles that of Amphioxus in all essential characters, but shows minor differences due largely to the greater amount of yolk present. In the first place, the blastoderm is no longer one layer of cells in thickness. Tangential divisions have increased the number of cells so that at the apical pole the blastoderm may be approximately four cells deep. Furthermore, the greater difference in size between the micromeres of the apical pole and the macromeres of the abapical pole results in an eccentric position of the segmentation cavity retained by the final blastocœle, which lies entirely within the limits of the apical hemisphere.

The chick. — The blastula of the hen's egg (Fig. 40) is difficult to homologize with the types just considered. The blastoderm is formed by the successive cleavages of the germinal disc and so

appears as a small island of micromeres on a large sphere of undivided yolk. Tangential or horizontal cleavages result in the building of several layers of cells, between the lowermost of which and the yolk there is a cleft-like yolk-cavity. It will be remembered that the radial cleavage planes never extend to the edge of the blastodisc. The marginal cells, therefore, are in connection with a ring of unsegmented protoplasm known as the **germ wall.** In this germ wall are found nuclei derived from the supernumerary sperm which entered the egg at the time of fertilization. These divide independently during the cleavage of the zygote, but finally degenerate completely. As cells are

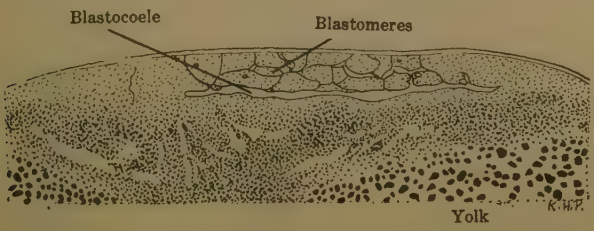


FIG. 40. — Section of blastula of chick, early. (After Patterson.)

added by tangential divisions to the central region of the blastoderm from the marginal part, the nuclei of the marginal cells finally are forced into the germ wall. Here they continue to divide but as no cleavage planes develop about them, the germ wall becomes converted into a **syncytium** (a mass of protoplasm containing many nuclei), which retains its connection with the yolk at its outer margin, as well as with the central cells of the blastoderm. Around those nuclei of the germ wall which lie nearest the blastoderm proper, cells are formed and added to the blastoderm. The blastoderm overlying the original yolk-cavity is known as the **area pellucida;** the marginal ring added from the germ wall is called the **area opaca.** The exterior margin of the germ wall advances out over the yolk to form the wall of the yolk sac (Fig. 56).

The mammal. — The blastula of the placental mammals is so atypical that it receives a different name, the blastocyst (Fig. 41). The cleavages by which it is formed are of the holoblastic

type, forming micromeres at the apical pole, macromeres at the abapical; but at the close of the segmentation period there is formed a solid mass of cells, known as a **morula,** without any trace of a segmentation cavity. In this morula one may distinguish a central mass of polyhedral cells and an enveloping layer of rounded cells which is known as the **trophoblast.** It is at this stage that the egg becomes attached to the wall of the uterus. The trophoblast now detaches itself from the central mass, or **embryonic knob,** except at a single point. The blastula of a placental mammal may be derived from the sauropsid type by the suppression of the yolk. The embryonic knob may be compared with the blastoderm of the chick, and the trophoderm with the germ wall. The cavity of the blastocyst is equivalent to the blastocœle of the chick.

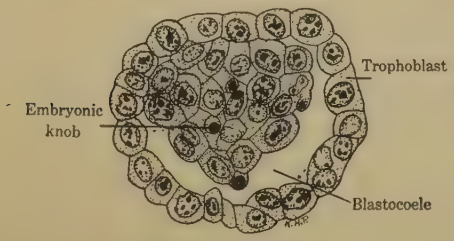


Fig. 41. — Section of blastocyst of bat. (After Van Beneden.)

B. THE FORMATION OF THE GASTRULA

The blastula is an embryo with a single layer, or mass of cells, which has arisen from the zygote by a series of cell divisions, accompanied by the localization of certain organ-forming substances. There has been little or no growth in mass. Aside from differences in size, due to the amount of contained yolk, the blastomeres are morphologically indistinguishable. The cavity of the blastula is not in communication with the exterior. The period that follows immediately upon the formation of the blastula is distinguished by growth and by differentiation accompanying the processes of division and localization.

Growth. — Growth depends on two principal factors: the imbibition of water, **hydration;** and the ingestion of food, **nutrition.** During the first two weeks after hatching, the larva of the frog increases its percentage of water from 56 to 96 per cent. In later development this percentage decreases. The chick after two days' incubation has 83 per cent of its body

weight as water, while after eleven days it has only 59 per cent. The human embryo is 97.5 per cent water at the age of six weeks, while at 39 weeks this percentage has fallen to 74.2. Nutrition during the embryonic period depends upon the amount of yolk present; during the larval or the fœtal period, nutrition depends upon extraneous sources of food, microscopic animals or plants on the one hand, foodstuffs in the maternal blood on the other.

Differentiation. — This growth on the part of the embryo is accompanied by a differentiation of the blastomeres into three strata, the **germ layers,** of which the outer layer is called the **ectoderm,** the middle layer the **mesoderm,** and the inner layer the **endoderm.** Each of these layers gives rise to definite organs in later development. Thus, the ectoderm, broadly speaking, gives rise to the epidermis and all its derivatives, the sense organs, and the nervous system; the endoderm forms the epithelial lining of the alimentary tract and all of its derivatives, including the respiratory organs; the mesoderm produces the bulk of the body, including the muscles, skeleton, vascular and urogenital systems. The details of the process of germ-layer formation are often obscure and confusing, as three different events are overlapping. The first of these is the conversion of the single-layered blastula into a two-layered gastrula. The second is the development from this of a three-layered embryo through the appearance of the mesoderm. Finally, the process is complicated by the precocious development of the axial notochord and neural tube, as well as the early stages of cephalization and metamerism.

The gastrula. — The process of gastrulation, by means of which the blastomeres become segregated into the two primary germ layers, ectoderm and endoderm, is modified by the amount and arrangement of the yolk. In oligolecithal eggs (Fig. 42*A*1–4) the blastula is transformed into a gastrula by the process of **invagination** whereby the macromeres of the abapical pole are pushed into the cavity of the blastocœle, obliterating it and forming a new cavity, the **gastrocœle.** The gastrocœle communicates with the exterior by a wide opening called the **blastopore.** The endoderm formed from the macromeres by invagination is called the **primary endoderm.** Even in oligolecithal eggs, invagination is sometimes accompanied by the process of **epiboly**

whereby local growth occurs at the margin of the blastopore at a point designated as the dorsal lip with reference to its position in the later embryo. The cells formed at this point segregate into ectoderm without and endoderm within, according to methods described in the paragraphs following. This later endoderm is called **secondary.** In lecithal eggs (Fig. 42*B*1–4), the greater

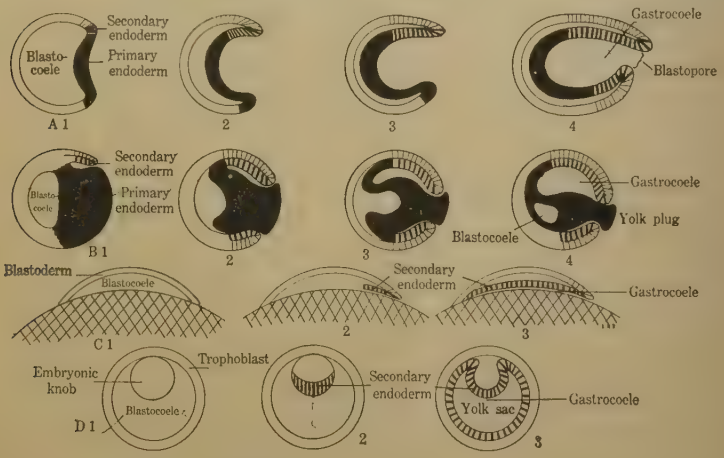


FIG. 42. — Diagram of gastrulation. Formation of endoderm and ectoderm (mesoderm omitted). A 1, 2, 3, 4, in Amphioxus. B 1, 2, 3, 4, in the frog. C 1, 2, 3, in the chick. D 1, 2, 3, in mammal.

part of the endoderm is formed by epiboly and is, therefore, secondary; but there is a certain amount of primary endoderm formed by the inward migration of small cells derived from the macromeres. In macrolecithal eggs (Fig. 42*C*1–3), gastrulation occurs entirely by epiboly, the endoderm is secondary, and the gastrocœle is a cavity with a floor of undivided yolk. Among the mammals the gastrula is formed by **delamination** from the ventral cells of the embryonic knob, which then migrate to line the cavity of the blastocyst (Fig. 42*D*1–3).

Amphioxus. — As in the formation of the blastula, the typical method of gastrulation occurs only in Amphioxus (Fig. 43). Here the lower hemisphere of the blastula, containing the yolk-laden macromeres, flattens, thus causing the blastocœle to assume a hemispherical shape (Fig. 39*a*). In this plate, not exactly

in the center but somewhat toward the side that later development shows to be dorsal, a depression is formed. This depression deepens and widens until the abapical hemisphere is completely contained within the apical hemisphere, and the blastocœle is flattened out. The contents of this cavity are expressed through the macromeres into the new cavity formed by this process of invagination, which is called the gastrocœle. Unlike its predecessor, this new cavity communicates with the exterior by means of a wide mouth, known as the blastopore.

The dorsal margin of the blastopore now becomes a center of local growth, comparable to the germ wall of the chick. Frequent

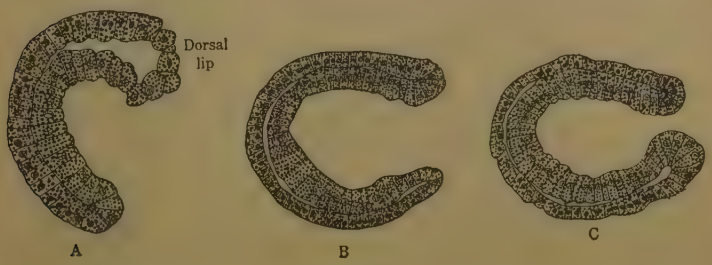


FIG. 43. — Sections showing three stages of gastrulation in Amphioxus. (After MacBride.)

mitoses take place, commencing at the margin nearest the point where the gastrular invagination began. Growth at this point results in the backward extension of the lip, a process known as overgrowth or epiboly, which brings about an addition to the gastrocœle. The cells formed at the gastrular lip separate into two groups, the more external of which are added to the ectoderm, while the internal group is added to the endoderm. To distinguish the latter from the endoderm formed by invagination, it is called the secondary endoderm. There are two theories as to the method by which the secondary endoderm is segregated from the cells formed at the gastrular lip: (1) by **involution**; (2) by **delamination** (or **gastrular cleavage**). According to the first theory, some of the cells formed at the growing edge of the gastrula roll inward and grow forward while the edge itself moves backward. According to the second theory, a cleft occurs in the

growing lip, which separates the outer cells, ectoderm, from the inner cells, endoderm.

The opening into the cavity of the gastrula grows constantly smaller as this process of epiboly continues. This seems to be

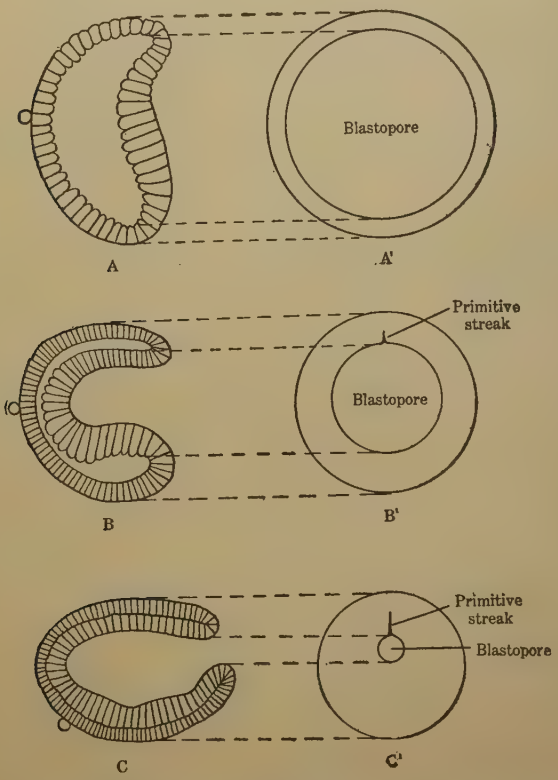


Fig. 44. — Diagrams of concrescence in Amphioxus. A, B, C, sagittal sections. A′, B′, C′, caudal views.

due to the right and left halves of the dorsal lip fusing together from the anterior end backward, thus adding to the length of the dorsal surface at the expense of the sides of the gastrular lip and, consequently, of the opening which it encloses. This phenomenon is known as **concrescence** (Fig. 44).

The frog. — In the frog blastula, the first indication of gastrulation is the appearance of a short horizontal groove at the boundary between the pigmented and non-pigmented hemispheres on the side where the grey crescent formerly appeared. Gradually the pigmented area grows down over the non-pigmented area, preceded by the groove, which deepens and lengthens, assuming first the form of a crescent and finally that of a small circle which completely surrounds the non-pigmented area. The groove moves from its original position about 25° below the equator almost 75° around the egg. Following this migration, the egg itself revolves about a horizontal axis in the opposite direction, through an angle of 100° (Fig. 45).

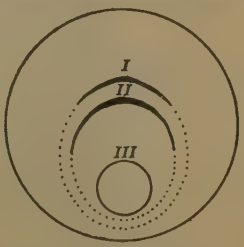


FIG. 45. — Diagram showing three stages in the formation of the blastopore in the frog's egg. (After Morgan.)

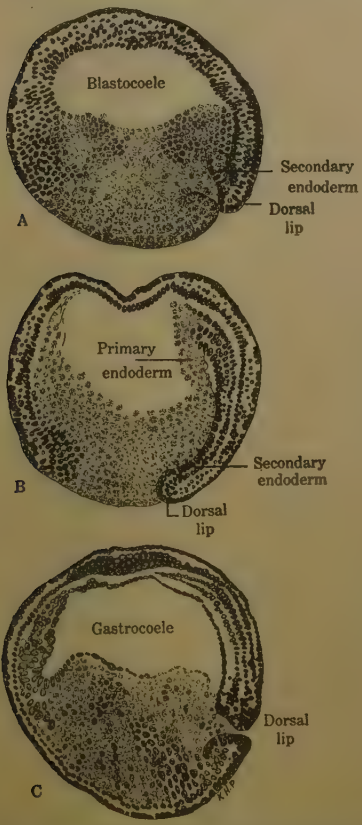


FIG. 46. — Sections showing three stages in the gastrulation of the frog's egg. (After Brachet.)

If a series of sagittal sections is studied (Fig. 46), it can be seen that this backward movement of the groove represents the actual overgrowth of the non-pigmented area by the pigmented micromeres. This is an example of epiboly. Furthermore, the cells formed at this

growing point separate again into ectoderm and endoderm. It is a debated point whether this takes place by involution or by delamination. In either case the groove must represent the dorsal lip of the gastrula and the endoderm formed at this point must be considered secondary. A similar epiboly and differentiation of ectoderm and endoderm take place at the lateral and ventral lips of the gastrula as these develop. But meantime changes are occurring in the large yolk-laden macromeres. Along the floor of the blastocœle, mitoses are taking place, and small cells, formed from the macromeres, push upward along the sides of the blastocœle until they meet at its roof. This is primary endoderm, for inward migration is the only sort of invagination

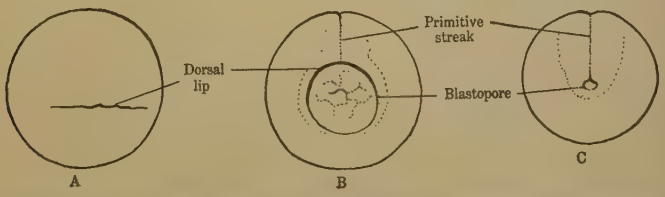


Fig. 47. — Three caudal views of Cryptobranchus eggs showing concrescence. (After B. G. Smith.)

possible in the large and heavy macromeres. Later the sides grow up beneath the secondary endoderm to form a new roof of the gastrocœle. This cavity enlarges at the expense of the blastocœle, which loses its contents into the younger cavity and eventually disappears. The gastrocœle does not communicate directly with the exterior, as the potential blastopore is filled with a mass of macromeres, the **yolk plug,** which is in direct connection with the macromeres of the thick floor of the gastrocœle. Finally the blastopore becomes reduced by concrescence until it eventually becomes but a small cephalocaudal slit (Fig. 47).

The chick. — The method of gastrulation in the chick departs still further from the type seen in Amphioxus. Here the blastocœle is only a narrow cleft between the blastoderm and the undivided yolk. The central part of the blastoderm, or area pellucida, represents the apical hemisphere of the oligolecithal or lecithal egg, while the peripheral germ wall represents the equatorial band of intermediate cells observed in these small-yolked

types. There are two main theories as to the method of gastrulation. The first, advocated by Duval and others, was that the ectoderm and endoderm were separated by a tangential split, or entirely by delamination. The second, supported by the observations and experiments of Patterson on the pigeon's egg, is that a gastrular lip is formed at the posterior end of the blastoderm.

This lip appears as a thinning of the blastoderm at the posterior end, accompanied by an increase in the diameter of the blastoderm and an interruption of the germ wall, so that at this point the thin sector of blastoderm lies directly on the yolk (Fig. 48). This free margin now begins to thicken, and at the same time endodermal cells migrate inward to form the lower germ layer. Patterson held that this migration commences as an involution of the free margin of the blastoderm. It is also possible that the origin of this proliferation lies in the delamination of the cells of the gastrular lip, as suggested in the case of the frog. In either case the endoderm so formed is clearly secondary, and the cavity is roofed by endoderm but with a floor of undivided yolk. The blastocœle is represented by the line of separation between ectoderm and endoderm. The blastopore is the space between the

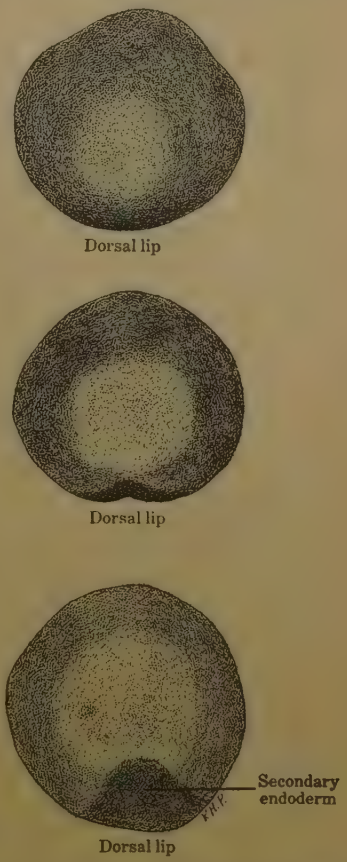


Fig. 48. — Surface views showing three stages in the gastrulation of the hen's egg. (After Patterson.)

gastrular lip and the yolk; it is soon obliterated by the concrescence of the germ wall from right and left (see page 84).

At the time the egg is laid, the contained embryo is composed of two layers, the ectoderm and endoderm, overlying the gastrocœle. The germ wall, which is in direct connection with the embryonic area at its inner margin, is connected with the yolk below by its syncytial portion, the **zone of junction,** and is growing out over the yolk at the **margin of overgrowth** (Fig. 57). There is no apparent blastopore, but (see page 84) there is a potential structure, now quiescent, which will develop in such a way as to merit that appellation.

The mammal. — In the placental mammals is found a method of gastrulation entirely devoid of a blastopore or gastrular lip. It will be remembered that, in place of the typical blastula, the mammal forms a blastocyst. In this the embryonic knob represents the blastoderm of the reptiles and birds, and a yolk-cavity is present. The ectoderm and endoderm are formed in the embryonic knob by delamination. The cells away from the point at which the knob is attached to the trophoblast undergo numerous mitoses and separate from the overlying cells as the endoderm. Later this layer extends upward around the knob and then grows down around the inside of the trophoblast to form a vestigial yolk sac (Fig. 49). The remaining cells of the trophoblast form the ectoderm, which later flattens out, in connection with the formation of the amnion (see next chapter). The two layers form a flat plate known as the **embryonic shield** (Fig. 55), which may be compared with the area pellucida of the chick.

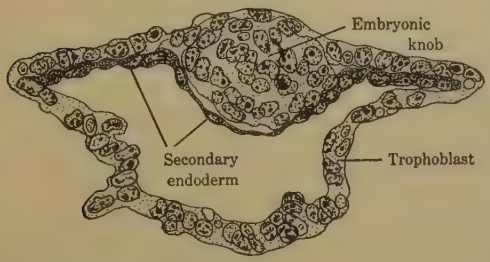


Fig. 49. — Section showing gastrulation in the bat's egg. (After Van Beneden.)

C. THE FORMATION OF THE MESODERM

The mesoderm, or middle germ layer, is destined to give rise to the great mass of the vertebrate body. It appears in three forms: (1) the axial **notochord;** (2) the lateral sheets of **mesoderm;** and (3) groups of wandering amœboid cells, known collectively as the **mesenchyme.** In many treatises the notochord is described as a derivative of the endoderm, but, as seen below, it arises in the same manner, from the same origin, and at the same time as the lateral sheets of mesoderm, and may

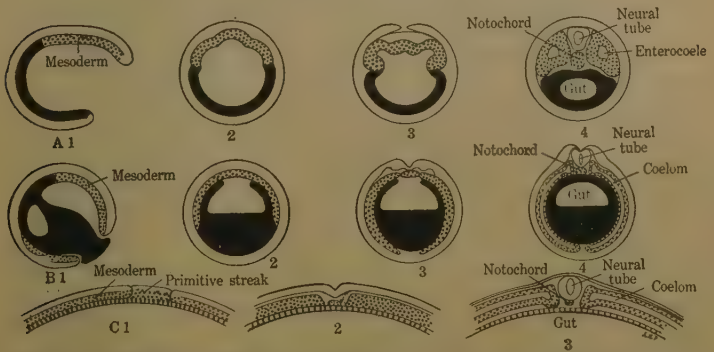


FIG. 50. — Diagrams of mesoderm formation. A 1, 2, 3, 4, in Amphioxus. B 1, 2, 3, 4, in the frog. C 1, 2, 3, in the chick. A 1, B 1, and C 1 are sagittal sections; the others are transverse.

properly be reckoned as a part of the middle germ layer, precociously differentiated in accordance with its importance in early embryonic life. It will be seen from the following paragraphs that in all cases the mesoderm originates entirely or in great part from the roof of the gastrocœle which in turn was formed from cells originating from the growing zone at the dorsal lip of the blastopore. In Amphioxus (Fig. 50*A*1–4), the cells destined to form mesoderm are carried in with the endoderm, to separate out later as the **enterocœlic pouches** and **notochordal groove.** In the frog (Fig. 50*B*1–4) the secondary endoderm formed at the dorsal lip becomes mesoderm. In the chick (Fig. 50*C*1–3) the endoderm is formed at the first appearance of the dorsal lip, the mesoderm at its renascence as the primitive streak. In the

placental mammal, the endoderm is formed by delamination, prior to the appearance of the primitive streak, which is the last vestige of the blastopore and has the single function of segregating the mesoderm.

Amphioxus. — In Amphioxus (Fig. 51) the roof of the gastrocœle forms three dorsal longitudinal grooves. The median groove is the primordium of the notochord; from the two lateral grooves the mesoderm originates. The folds of the median groove grow ventrad and fuse, forming a transitory **notochordal canal,**

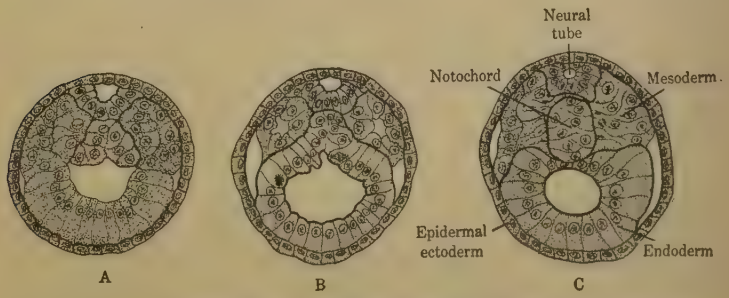


FIG. 51. — Transverse sections illustrating mesoderm formation in Amphioxus. (After Cerfontaine.)

the lumen of which is soon occluded to leave a solid axial rod, the notochord. Transverse constrictions meantime appear in the lateral grooves, dividing them into a series of pockets, the enterocœlic pouches. The constrictions continue and eventually pinch off these pouches into closed sacs, called **somites;** from these the mesoderm proper is produced.

The frog. — In the frog (Fig. 52) the notochord and part of the mesoderm proper are likewise formed from the roof of the gastrocœle. In this embryo, however, the process of mesoderm and notochord formation is advanced until it is almost synchronous with gastrulation. The secondary endoderm formed at the dorsal lip of the blastopore separates into three cephalocaudal strips, of which the median one gives rise to the notochord, while the lateral ones grow forward and downward as great mesodermal sheets. To these sheets there are added other masses of mesoderm, delaminated *in situ* from the primary endoderm and from the secondary endoderm formed at the lateral

and ventral lips of the blastopore. At the end of this process there is a continuous layer of mesoderm, uninterrupted save by the axial notochord at the dorsal surface, and by the blastopore at the posterior end. At the margin of the blastopore all three germ layers merge into a growing zone of undifferentiated cells

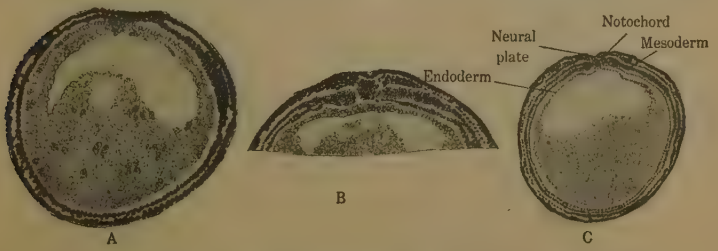


Fig. 52. — Transverse sections illustrating mesoderm formation in the frog. (After Brachet.)

which later are added to one or another of the layers. Later, the mesoderm becomes divided, by transverse constrictions, into somites.

The chick. — In the chick, the mesoderm is formed in connection with a structure known as the **primitive streak** (Fig. 53).

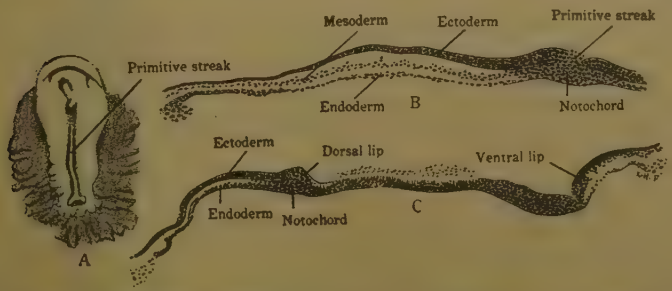


Fig. 53. — Mesoderm formation in the chick. A, surface view. B, transverse section across primitive streak. C, sagittal section.

This is an axial thickening of the ectoderm in the posterior two-thirds of the area pellucida, formed during the first half day of incubation. In it there is developed a shallow groove called the **primitive groove,** bounded by the **primitive folds.** At the anterior end is a thickening, the **primitive knot,** followed by a

depression, the **primitive pit.** From the primitive knot there grows forward a strip of tissue (called the **head process**), which later becomes the notochord. From the sides and posterior end of the streak, the mesoderm proper grows out to form a shield-shaped middle layer interrupted only by the streak itself and the notochord in front. The primitive streak represents a growing area which corresponds to the gastrular lip. The primitive groove then represents a blastopore which never becomes connected with the endoderm, except, in rare instances, by an extension of the primitive pit, which in some birds opens

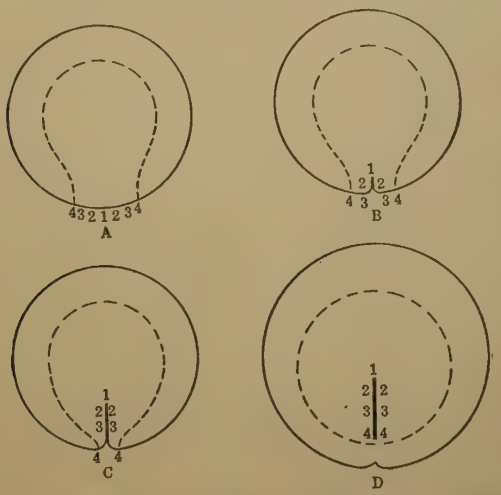


FIG. 54. — Diagrams of concrescence in the chick. Four stages. (After Lillie, modified.)

into the gastrocœle. In order to account for this transformation of a crescentic dorsal lip into an axial streak, the theory of concrescence is again invoked (Fig. 54).

It will be remembered that the gastrular lip has already appeared once as a transitory region of local growth, by means of which endoderm formation originated (see page 79). Thereafter it was obliterated by the ingrowth of the germ ring from right and left and the local growth ceased. Now, if this concrescence of the germ ring continues, the original gastrular margin will be folded together and left behind as an axial streak representing the fused

right and left halves of the dorsal lip. If local growth is renewed at this region we shall have a proliferation of cells such as is seen in the activity of the primitive streak.

It is of interest to observe that concrescence of this type, whereby the crescentic dorsal lip of the blastopore becomes transformed into a fused primitive streak, is easily demonstrable in such forms as the gymnophiona (legless amphibia).

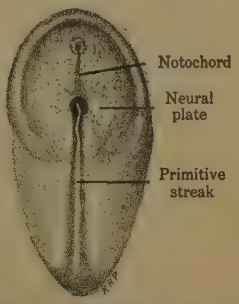


FIG. 55. — Surface view of embryonic shield of rabbit, showing primitive streak. Compare Fig. 53*a*. (After Van Beneden.)

The mammal. — The method of mesoderm formation in the mammals is essentially similar to that of the chick. In the embryonic shield, formed by the flattening out of the ectoderm and endoderm (Fig. 55), and comparable to the area pellucida, there forms a primitive streak with groove, folds, knot, and pit. This pit, however, frequently opens through into the potential gastrocœle. From the primitive streak the notochord and mesoderm proper grow out exactly as in the bird.

D. EARLY DIFFERENTIATIONS IN THE GERM LAYERS

The processes of endoderm formation and the formation of the mesoderm are further complicated by the early differentiation of the germ layers into the primordia of the various organ systems. This differentiation is often advanced so that it takes place concomitantly with the formation of the layers. In this account the immediate differentiations of each germ layer are discussed separately, notwithstanding the fact that in the embryo many of these processes occur practically simultaneously.

The ectoderm. — The principal differentiation of the ectoderm is that which results in the separation of the nervous and sensory ectoderm from the **epidermis** proper. This is inaugurated by the flattening of the ectoderm in front of the blastopore to form a **neural plate** (Fig. 50). The edges, principally the lateral edges, grow upward to form the **neural folds,** while the median groove, so developed, grows downward as the **neural groove.** The last stage in this process is brought about by the union and fusion of the neural folds, whereby the neural groove is trans-

formed into a **neural tube** which loses connection with the overlying ectoderm. The cavity of this tube is the **neurocœle,** and the openings at the anterior and posterior ends are the anterior and posterior **neuropores,** respectively. At the posterior end the union of the neural folds roofs over the blastopore so that this opening of the gut (enteron) becomes continuous with the posterior neuropore by means of an extension of the neurocœle known as the **neurenteric canal** (Fig. 76).

Amphioxus. — In Amphioxus (Fig. 51) is seen a variant of this process, inasmuch as the neural plate becomes discontinuous with the non-nervous ectoderm and two sets of folds are formed. The inner ones, formed from the neural plate, are the neural folds which fuse to form the neural tube, while the outer ones, formed by the non-nervous ectoderm, may be called the **epidermal folds,** by which the epidermis grows together over the neural tube.

The frog. — In the frog (Fig. 52) the ectoderm becomes delaminated into an outer pigmented epidermal layer and an inner nervous layer. Cross sections of the neural plate show that, within this area, local growth of the nervous layer produces five longitudinal strips: (1) a median thin strip, (2) two internal lateral thick strips, and (3) two external lateral thick strips. The neural folds involve both lateral strips and the outlying margin of the non-nervous ectoderm. The median strip becomes the floor of the neural tube; the internal lateral strips become the sidewalls; the external lateral strips are carried up to form neural crests which will lie on either side of the completed neural fold. The outer non-nervous portions of the folds fuse to become the dorsal epidermis as the neural tube withdraws beneath the surface. Neuropores and a neurenteric canal are formed according to the general method already described.

The chick. — In the chick (Figs. 156, 157) a neural plate forms in front of the primitive streak, and, at the edge of this, neural folds develop, involving both the margin of the plate and the epidermal ectoderm bounding it, as in the case of the frog. The folds fuse from the anterior end backward, leaving a typical anterior neuropore and a widely open posterior neuropore known as the **rhomboidal sinus.** The neural folds ultimately enclose the primitive streak, and for this reason the primitive pit has sometimes been homologized with a neurenteric canal. This

homology seems rather far-fetched; the interpretation of the primitive pit as the last vestige of the blastopore is a preferable one.

The mammal. — In the mammal, conditions are similar to those in the chick, except for the fact that an open primitive pit is more common and this is sometimes extended into the head process as the **notochordal canal.**

The mesoderm. — In the early differentiation of the mesoderm (Fig. 56) three different regions are indicated in the mesoderm on either side of the notochord. These are, commencing with the most internal division, the **epimere, mesomere,** and **hypomere.** The epimere becomes divided by transverse constrictions into metameric blocks called **somites,** which give rise to muscles, vertebræ, and part of the dermis. The mesomere, throughout part of its length, undergoes similar segmentation into blocks known as **nephrotomes,** which give rise to the nephric organs and to the gonads. The hypomere, on the contrary, undergoes a tangential division by means of which two layers are formed, the outer **somatic** mesoderm, which comes in contact with the epidermis and forms the greater part of the dermis and some muscles, and the inner **splanchnic** mesoderm, which joins the endoderm and forms the outer lining of the organs of endodermal origin. The somatic mesoderm and ectoderm are often called collectively the **somatopleure,** and the splanchnic mesoderm and endoderm are known as the **splanchnopleure.** Sometimes cavities occur in the myotomes: these are called **myocœles.** Cavities formed in the nephrotomes are **nephrocœles.** The great cavity lying between the somatopleure and the splanchnopleure is the **cœlom.**

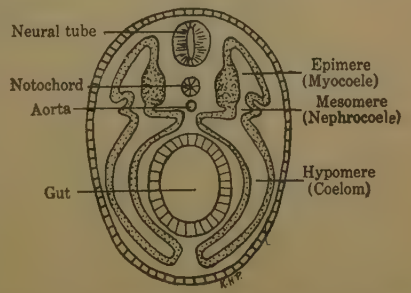


FIG. 56. — Diagrammatic cross section to show divisions of the cœlom.

Amphioxus. — In Amphioxus, the mesoderm early forms enterocœlic pouches (Fig. 51). These become constricted off

from the enteron, and grow both dorsally and ventrally. The dorsal portions are the **myotomes.** The ventral portions coalesce to form the cœlom. No nephrotomes are formed, and the gonads develop from the anterior lower angles of the myotomes. They grow forward into the posterior lower angles of the myotomes in front of each gonad, thus forming **gonocœles** which later are constricted away from their proper myotomes, so that each gonad is contained in a gonocœle derived from the myotome immediately preceding it.

The frog. — In the frog (Fig. 52), the first division of the mesoderm occurs as a longitudinal constriction separating the epimere or **vertebral plate,** as it is often called, from the hypomere, or **lateral plate.** The epimere divides into somites, and the lines of cleavage separating these continue into the narrow **intermediate mass** which represents the mesomere, and divide it into nephrotomes. The hypomere separates into the somatic and splanchnic layers, and this delamination continues through mesomere and epimere so that there are three sets of continuous cavities, the myocœles, nephrocœles, and cœlom. The last-named cavity extends completely around the sides and ventral surface of the embryo. Later, the myocœles and nephrocœles are cut off from the cœlom, and the myocœles disappear completely.

The chick. — In the chick, the embryo is still a disc upon the great yolk mass, extending over the latter by the activity of the margin of overgrowth. This margin develops around the yolk mass, and as it does so three regions are formed. The outer region includes the margin of overgrowth and the zone of junction, **area vitellina externa;** the middle zone is that part of the germ wall into which the mesoderm has not yet penetrated, **area vitellina interna,** while the inner zone is that in which the germ wall has become differentiated into an overlying mesoderm and an underlying endoderm, which together form the splanchnopleure of the yolk sac. This zone is the **area vasculosa,** so called because within it the extra-embryonic blood vessels will develop (Fig. 57).

In the **embryonic region,** vertebral, intermediate, and lateral plates are formed as in the frog. The vertebral plates are divided into myotomes in which a transitory myocœle develops; the intermediate plate, however, does not segment, nor do

nephrocœles develop. The lateral plate delaminates into the somatic and splanchnic layers, enclosing a cœlom, which ex-

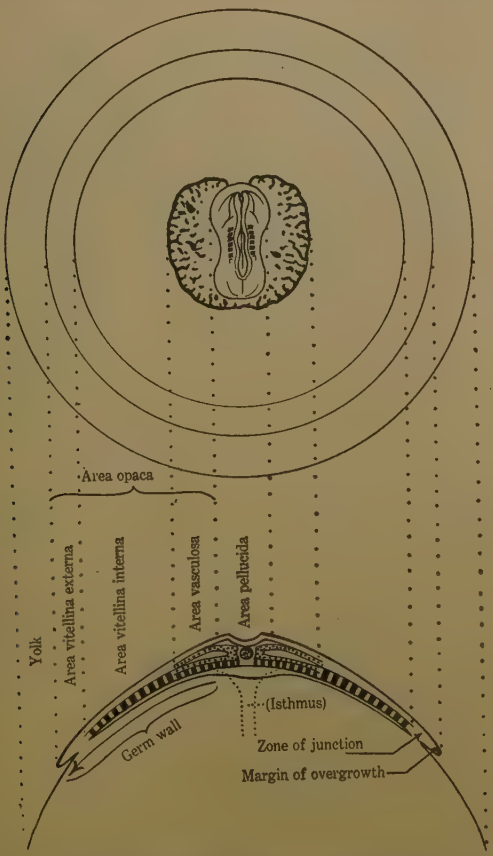


FIG. 57. — Diagrams of extra-embryonic areas of chick. Above, surface view. Below, transverse section.

tends from the intermediate plate to the external boundary of the area vasculosa. This will later become divided into the embryonic cœlom, or body cavity of the embryo, and the extra-embryonic cœlom or **exocœle.**

The mammal. — In the mammal, vertebral, intermediate, and lateral plates are formed, the first and second of which become segmented into myotomes and nephrotomes, respectively. The myotomes have a transient myocœle. The lateral plate forms the cœlom, which later becomes confluent with the exocœle, as will be shown in the following chapter.

The mesenchyme. — This is the term applied to the amœboid cells that detach themselves from existing cell aggregates and wander through the body, reproducing by mitosis and acting as semi-independent units. Frequently these cells mass together and give rise to new aggregates, such as connective tissues, cartilage, bone, smooth muscles, cardiac muscles, and the endothelia of the blood vessels. Other mesenchymal cells, such as the leucocytes, retain their identity.

Some confusion arises from the fact that cells from other germ layers, as, for example, in the neural crests, present a mesenchymatous appearance. The primordial germ cells, for example, appear first in the endoderm, whence they migrate, like amœboid cells, into their definitive position in the gonads. But, although mesenchymatous cells may appear from any germ layer, we shall restrict the term mesenchyme to the wandering cells that originate from the mesoderm. The most prolific sources of mesenchyme production are (1) the epimere anterior to the somites, which produces the mesenchyme of the head region; (2) the internal ventral angles of the somites, often known as the sclerotomes, which produce a great part of the skeleton; and (3) the splanchnic mesoderm, which gives rise to most of the vascular system.

The endoderm. — As compared with the other germ layers, there is little early differentiation of the endoderm. The only apparent activity is that involving the completion of the enteron, or digestive cavity, and its subsequent division into three regions: the **fore-gut,** in front of the yolk stalk; the **mid-gut,** co-extensive with the yolk stalk; and the **hind-gut,** behind the yolk stalk (see Chapter IV).

Amphioxus. — In Amphioxus (Fig. 51), the roof of the gastrocœle is consumed in mesoderm formation. This gap in the continuity of the digestive cavity is replaced by the upward growth of endoderm from the sides of the cavity, which fuse beneath the notochord to supply a new roof.

The frog. — As growth continues, the yolk in the thick floor of the gastrocœle is consumed by the developing frog embryo, with the result that the floor finally becomes as thin as the roof and sides, while the yolk plug is resorbed in this process.

The chick. — In the chick, the roof of the gastrocœle is formed by secondary endoderm, while the floor of this cavity is supplied by the undivided yolk. The sides of the embryonic endoderm grow together to form a floor at the anterior and posterior ends, to form a fore-gut and hind-gut, respectively, while the middle region remains open. The details of this process will be discussed in Chapter V.

The mammal. — The endoderm of the mammal originates in connection with the formation of the yolk sac, which will be considered in Chapter IV.

E. THE SIGNIFICANCE OF THE GERM LAYERS

The blastula and gastrula stages present several problems of great interest to the theoretical embryologist. From the com-

Fig. 58. — Diagrams of "Blastæa" (A), and "Gastræa" (B). (After Lang.)

parative point of view the question has been raised as to the significance of these stages in evolutionary history. The experimentalist has been more interested in learning how the different

organ-forming substances are distributed during the period of germ-layer formation and how rigidly these layers are restricted with respect to the structure to be formed from them.

The "blastæa" and "gastræa" hypothesis. — Hæckel considered that the blastula and gastrula represent ancestral stages in the phylogeny of all forms displaying these ontogenetic stages. As he was unable to find any living animals exactly comparable he was compelled to postulate two hypothetical ancestors, the blastæa and the gastræa respectively. The **blastæa** representing the blastula of the Amphioxus type resembled the Volvox type of colonial protozoa. The **gastræa** deduced from a gastrula like that of Amphioxus, he described as similar to the simplest cœlenterates. Lang, however, has pointed out that both Volvox and the cœlenterates possess more different types of cells than Hæckel took into account and his diagrams of the hypothetical blastæa and gastræa are reproduced in Fig. 58.

Organ-forming substances. — Recent investigations by means of staining regions of the blastula with intra-vitam (non-lethal) dyes have shown that it is possible to follow the later history of the stained regions and thus to demonstrate that the blastomeres segregated in germ-layer formation are already differentiated in the blastula stage. In the case of Triton, a caudate amphibian, Vogt has shown that most of the surface material turned in at the dorsal lip of the blastopore (secondary endoderm) becomes mesoderm, giving rise to both the notochord and the mesoderm proper. The first material so turned in however becomes the lining of the fore-gut, while the last gives rise to the mesoderm of the tail. Of the remaining surface material more than half becomes the neural plate, leaving only approximately a quarter of the original surface of the blastula as the ectoderm of the skin (Fig. 59).

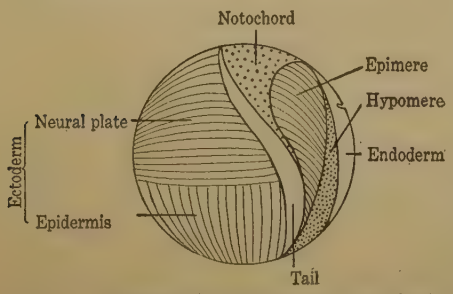


Fig. 59. — Diagram of a Triton embryo just beginning gastrulation. Side view. (After Vogt.)

Organizators. — Spemann has shown by grafting experiments that the materials anterior to the dorsal lip of the blastopore in amphibia act as an **organizator** or center of influence essential to the further development of the embryo. This material corresponds to that in the region of the grey crescent (Fig. 14) which according to Morgan gives indication of more rapid development even during cleavage. Spemann has also shown that the invagination of the neural plate (page 85) is induced by the presence beneath it of the secondary endoderm ("chorda-mesoderm") which acts as an organizator to this process.

The specificity of the germ layers. — Although it has been amply demonstrated that organ-forming stuffs have been segregated in the formation of the germ layers, and indeed long before this time, it must not be supposed that this differentiation is irreversible. The history of isolated blastomeres (page 67) is one indication to the contrary. Numerous experiments on grafting and regeneration also show that the fate of the individual blastomeres is largely determined by their final position in the developing embryo. Three sets of factors are involved in the history of the individual blastomere, first the genes in the nucleus, second the organ-forming stuffs in the cytoplasm, and finally the stimuli from its environment, including the surrounding blastomeres. The reactions resulting are surely complex and numerous enough to account for subsequent differentiation.

Summary. — We have now reviewed the steps by which the early embryo is formed from the fertilized egg. In the first period the zygote is divided into many blastomeres which resemble each other except in size, but which have different organ-forming substances. Following different patterns of cleavage according to the amount of yolk present, this period terminates with the formation of a blastula, which again presents different appearances according to the amount and distribution of yolk.

In the second period the blastomeres are rearranged into the germ layers, from which the organ systems are to be formed. During this period three separate sets of phenomena are distinguished: (1) gastrulation, or the formation of the primary germ layers, ectoderm and endoderm, involving in varying degrees invagination or epiboly according to the amount of yolk present; (2) the formation of the mesoderm, including the notochord and the mesenchyme, and associated with the

closing of the blastopore; and (3) the establishment of the primordia of the organ systems, which carries us over into the period of organogeny.

REFERENCES

Assheton, R. 1916. Growth in Length.
Brachet, A. 1921. Traité d'Embryologie, Books 3, 4 and 5.
Cowdry, E. G. 1924. General Cytology, Chap. 9.
Graham-Kerr, J. 1919. Textbook of Embryology, Vol. II, Chap. 1.
Hertwig, O. 1906. Handbuch, Vol. I, Chaps. 2 and 3.
Jenkinson, J. W. 1913. Vertebrate Embryology, Chaps. 5 and 6.
Kellicott, W. E. 1913. General Embryology, Chaps. 7 and 8.
Korschelt and Heider. 1902. Lehrbuch, Chaps. 7 and 8.
Lang, A. 1912. Handbuch der Morphologie der Wirbellosen Tiere, pp. 132–150.
MacBride, E. W. 1914. Textbook of Embryology, Vol. I, Chap. 17.
Morgan, T. W. 1927. Experimental Embryology. Chaps. 13 and 19.
Patterson, J. T. 1909. Gastrulation in the Pigeon's Egg, Journal of Morphology, XX, 1.
Wilson, E. B. 1925. The Cell, Chaps. 13 and 14.

CHAPTER IV

EMBRYONIC FORM AND MEMBRANES

After the formation of the germ layers, the early embryo already possesses the primordia of some of the principal organs. But before tracing the continued differentiation of these primordia in organogeny, we must examine the manner in which the general plan of the body is molded from the material differentiated up to this point, **morphogenesis.** Closely related to this is the process of **adnexogenesis,** the development of certain extra-embryonic membranes and appendages, which play particular rôles in embryonic (and fœtal) life but are discarded by the adult.

A. BODY FORM AND THE YOLK SAC

One of the principal factors in the early morphogenesis of the embryo is the amount and distribution of the yolk, which, after the formation of the germ layers, comes to lie in a sac of great or small capacity, known as the **yolk sac.** This is indistinguishable in oligolecithal types like Amphioxus. In lecithal types, like the frog, the yolk sac is merely a bulge on the ventral side of the embryo about the middle of the body, caused by the yolk in the floor of the gut (Fig. 60*A*). In the chick and all other macrolecithal types, the embryo develops from a plate floating on a vast mass of yolk which becomes enclosed in a gigantic yolk sac by the downward growth of the extra-embryonic splanchnopleure (Fig. 60*B*). Atypical oligolecithal embryos, such as those of placental

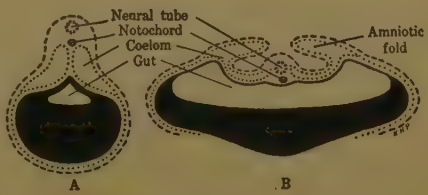


FIG. 60. — Diagrams to show influence of yolk on body form. Cross sections. A, small-yolked form like frog. B, large-yolked form like chick. (After Assheton.)

mammals, form a small yolk sac by different methods. These yolk sacs are functional for but a short time, although they may persist as rudimentary structures.

Growth in length. — In discussing the formation of the germ layers, it was pointed out that the gastrula was formed by two processes: the first occasioned by inward growth (invagination, or immigration) from the abapical hemisphere; the second occasioned by the backward growth of the gastrular lip (epiboly), accompanied by a differentiation of this added tissue through delamination or involution. There are two theories concerning further growth in length.

Concrescence. — The first of these is the theory of concrescence enunciated by His. This theory has already been used to explain the closure of the blastopore. By many it has been

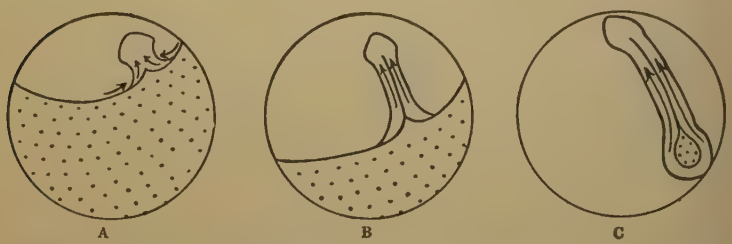


FIG. 61. — Diagrams to show growth in length by concrescence. Three stages. (After Assheton.)

extended to include the formation of the body, with the exception of the tail, by the coalescence of the two halves of the germ wall, each half of this wall corresponding to one half of the embryo, right or left (Fig. 61).

Notogenesis. — On the other hand, others, notably Assheton, have vigorously denied the existence of concrescence and maintained that growth in length is due solely to **notogenesis,** or the continued backward growth of the dorsal lip of the gastrula in anamniota, or of the primitive streak in amniota (Fig. 62).

Experimental evidence may be obtained by marking regions of the germ ring and observing the position of the marked portion in the older embryo. This evidence seems to support the conclusion that in the small-yolked types, like Amphioxus and the

frog or mammal, there is very little concrescence, whereas in large-yolked forms, like the dog-fish, trout, or chick, concrescence plays a considerable part in the growth of the embryo in length. In so far as the necessity of concrescence in explaining the bilateral symmetry of the vertebrate is concerned, it should be remembered that bilateral symmetry is, like polarity, often foreshadowed in the egg at the time of fertilization or even before.

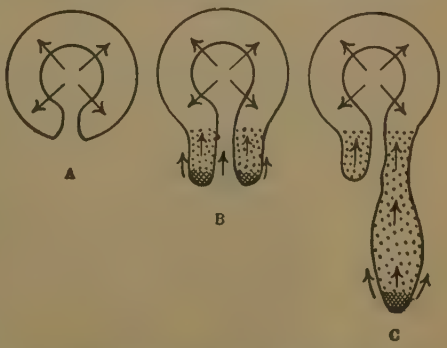


FIG. 62. — Diagrams to show growth in length by notogenesis. A, gastrula. B, epiboly at blastopore. C, notogenesis. (After Assheton.)

Metamerism. — With growth in length is associated a second factor in the development of the vertebrate body, that of **metamerism.** This is first indicated by the appearance of metameres, such as enterocœlic pouches in Amphioxus, and somites, etc., in the true vertebrates. In organogeny are found further evidences of metamerism in the nervous system, nephric system, vascular system, and others. All of these, however, develop in adaptation to the primary metamerism shown in the mesoderm. These metameres are formed successively, commencing with the anterior end, and therefore afford a basis of classifying the embryos in early stages of any species by the number of these units formed.

The metamerism in the vertebrates has afforded a basis for comparison with that of other metazoa, notably the annelid worms, and attempts have been made on this basis to postulate an " annelid ancestor " of the vertebrate stock.

Correlative with the backward formation of the metameres is their gradual disintegration in the anterior end of the body. But the discussion of this loss of metamerism carries over into the larger question of the formation of the head.

Cephalization. — Among other points, the vertebrates are characterized by the presence of a sharply demarcated anterior

region containing the mouth, the visceral arches, the special organs of sense, and the more specialized portion of the neural tube, the brain. In Amphioxus, however, this region is not highly developed. In vertebrate morphogenesis the head is the first region to be differentiated. The first indication of the neural folds and the first point at which they later fuse is invariably in this region; and in the large-yolked forms, like the chick, the head is the first part of the embryo to be freed from the underlying yolk.

The frog. — The formation of the head in the frog commences in the region of the embryo in front of the yolk mass. There a mouth develops by the formation of a ventral pit, the **stomodæum,** in a region previously localized by the fact that no mesoderm develops between ectoderm and endoderm. This pit breaks through the oral membrane of the primary germ layers, which separates it from the fore-gut, and so brings the enteric cavity from a sac-like condition to that of a tube. Just behind the stomodæum is a V-shaped groove which will become the **mucous gland,** or **"sucker."** From the ectoderm just external to the neural folds, an elevation appears, known as the **sense plate,** which extends in front of the neural tube and part way down each side, eventually merging with the neural folds on the dorsal surface. From this sense plate are differentiated the individual **placodes** of the organs of special sense, namely, the **olfactory placodes** of the nose, the **lens placodes** of the eyes, and the **otic placodes** of the ear. Behind the sense plate and separated from it by a slight constriction, is the **gill plate,** which becomes divided by five transverse furrows, the **visceral grooves,** into six **visceral arches.** The first of these, the **mandibular** arch, will give rise to the **jaws;** the second, to the **hyoid apparatus;** while on the third, fourth, and fifth, **external gills** develop. At this time the embryo hatches and may thereafter be called a larva. In connection with the head it should be noted that, in the early period of larval life, the first four visceral grooves extend inward until they establish connection with corresponding evaginations from the fore-gut, the **visceral pouches,** to form the **visceral clefts.** On the margins of these, **internal gills** develop, while the external gills become resorbed. Finally, a flap of ectoderm from the second arch on each side grows back over the gills, forming

the **opercula,** of which the one on the right closes completely, while that on the left remains open, as the **opercular aperture.** This condition is characteristic of the tadpole and persists until the metamorphosis of the larva into the adult form (Fig. 63*A*).

The chick.— In large-yolked forms like the chick, the head is formed by local growth, which results in a fold of the blastoderm, the **head fold,** accompanied by a depression beneath which

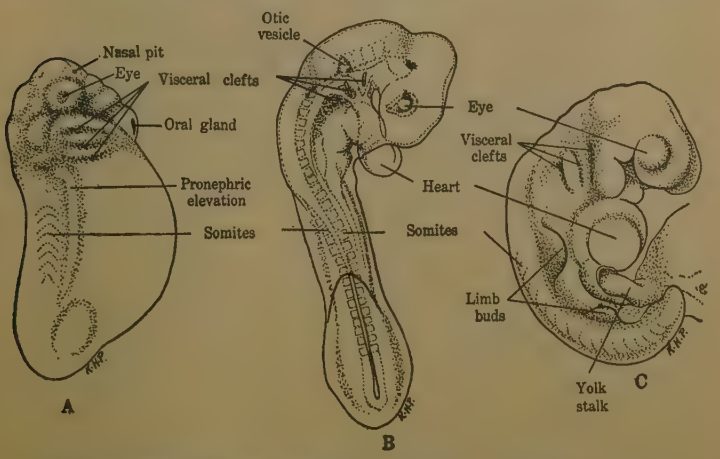


FIG. 63. — Diagrams of early embryos. A, of frog. B, of chick. C, of man. (A, after Patten; B, after Kerr; C, after His. All modified.)

is the **subcephalic pocket.** The folding process continues backward on both sides, gradually fusing in the median line and lifting the head from the yolk. The enclosed endoderm is the fore-gut, which is increasing in length at the expense of the open mid-gut. Even prior to the formation of the head fold, the neural folds have appeared in this region. The sense placodes, mouth, and visceral arches and grooves appear as they did in the frog, with the following exceptions: no sense plate or gill plate can be distinguished, and no external gills are formed, although vestigial gill filaments have been described. Only the first three furrows open into the fore-gut, and these never develop internal gills (Fig. 63*B*).

Man. — In man, the head fold and subcephalic pocket are indicated, the mouth opens, sensory placodes are formed, and

four visceral furrows and five arches are formed as in the chick; but none of the furrows open into the fore-gut (Fig. 63*C*).

Cervical flexure. — In both the chick and man, the head is inclined ventrally at the region of the visceral arches. This cervical flexure causes a construction which is the primordium of the **neck,** a characteristic region found only in amniotes.

Origin of the head. — There are many evidences that some degree of metamerism formerly existed in the head, shown by the arrangement of the cranial nerves, the formation of the eye muscles, and the existence of fugitive myotomes and head cavities. This metamerism has been lost with the enlargement and loss of flexibility of the brain. There have been many attempts to solve the problem of the number of metameres which go to make up the vertebrate head. Unfortunately, there is no unanimity, the number suggested varying from three to eighteen or more.

The tail. — This region of the body is distinguished from the head and trunk by the fact that the digestive tube and cœlom are absent.

The frog. — In the frog, the tail originates from two caudal buds, one on each side of the blastopore. As these buds grow backward they press against each other and flatten out to form the deep, thin tail of the larva. On the ventral side of the trunk, just anterior to the tail, is formed a pit known as the **proctodæum,** separated from the enteric cavity by a **cloacal membrane** of ectoderm and endoderm, which later ruptures to form the **cloacal opening.** This represents the ventral end of the blastopore.

The chick. — In the chick the tail arises by a **tail fold,** which represents the final union of the lateral extensions of the head fold. There is a **subcaudal pocket** which invaginates beneath the embryo more rapidly than the similar depressions beneath the **lateral folds,** so that the tail is separated from the yolk in the same general manner as the head. Consequently, the open mid-gut is reduced, while the fore-gut and hind-gut are enlarged, and the narrow connection between the enteric cavity and the yolk sac becomes the **yolk stalk.** A proctodæum is formed, which breaks through to form the cloacal opening.

Man. — In man a tail fold is formed and persists during the earlier part of fœtal life. It is later overgrown and resorbed.

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A yolk stalk is formed but loses its connection with the enteric cavity early in fœtal life. The proctodæum gives rise to the urogenital and anal openings.

The appendages. — The paired appendages generally characteristic of vertebrates arise as two pairs of **limb buds** (Fig. 63). In the chondrichthyes and osteichthyes these develop into fins; in all the tetrapoda, however, they form legs, sometimes especially modified as the wings of birds or the human arms. Even in forms like the snakes, which have lost the paired appendages, these limb buds make a transitory appearance.

In the frog, the posterior appendages are seen first, as the anterior appendages, which really are the first to form, are hidden beneath the opercula. At the time of metamorphosis the anterior appendages push out and the tail is resorbed. In the chick, the anterior pair of appendages are the first to appear as well as to develop. The same is true of man.

The yolk sac. — The method of formation of the yolk sac differs in large-yolked and small-yolked forms, for this structure is a part of the body in the latter case, and an extra-embryonic appendage in the former. Furthermore, the method of formation differs in the anamniota and the amniota.

The frog. — In the anamniota the yolk sac is composed of all three germ layers, as in the frog.

The chick. — In the amniota, on the other hand, the interposition of an exocœle between the somatic and splanchnic layers of the mesoderm results in the wall of the yolk sac being limited to the endoderm and splanchnic mesoderm alone.

The mammals. — In the placental mammals, where the yolk sac is really a vestigial structure, two different methods of formation are utilized. In the majority of cases, the endoderm, delaminated from the ventral side of the embryonic knob, grows out over the embryonic knob and then around the inner side of the trophoblast to form the yolk sac. In some animals, like the guinea pig, the embryonic knob hangs down into the cavity of the blastocyst, so far that in cross section the ectoderm lies inside the endoderm. This apparent anomaly was known to the older embryologists as **entypy** (Fig. 64). In other mammals, such as man, the formation of the rudimentary yolk sac commences as a vesicle inside the delaminated endoderm, of which the roof be-

comes the enteric endoderm. The human yolk sac becomes incorporated in the umbilical cord and later loses its connection with the gut, whereas in most other vertebrates the yolk sac is resorbed within the gut.

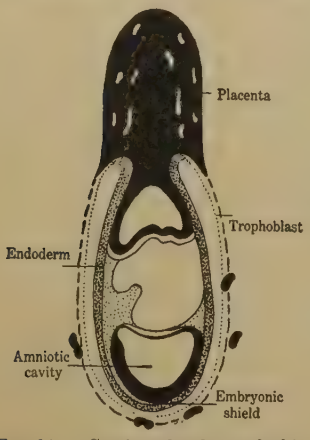

FIG. 64. — Section of embryo of white rat, to show entypy. (After Grosser.)

The area vasculosa. — As the yolk sac is the primitive store house for embryonic food, there arises on its surface a network of blood vessels forming the area vasculosa, by means of which blood is transported to and from the embryonic body. The yolk sac also takes on other functions. In some viviparous chondrichthyes, the yolk sac is pressed against the oviduct and its vessels absorb the "**uterine milk**" which is secreted by the walls of the oviduct much as is a tertiary egg membrane (*cf.* the albumen of oviparous forms). A similar "**yolk-sac placenta**" is found among the marsupial mammals.

B. AMNION AND CHORION

Most of the anamniote vertebrates lay their eggs in a fluid medium in which they hatch and assume the larval condition. In such a medium the eggs are not susceptible to violent changes in temperature or humidity, or to mechanical injury. The tertiary egg membranes in which they are wrapped often protect them from being eaten. The amniota, however, either lay their eggs under terrestrial conditions or retain them within the maternal body for a considerable period. These eggs are protected by two membranes known as the **amnion** and **chorion.**

The amnion, or inner membrane, is formed of somatopleure, which is continuous with the somatopleure of the embryo. It contains an **amniotic fluid** in which the embryo floats as in a waterbath, attached by the umbilicus to the yolk sac. The chorion, or outer membrane, is likewise formed of somatopleure and is continuous with the amnion at a point above the embryo

known as the **sero-amniotic raphe.** The cavity of the chorion is continuous with the cœlom and is, therefore, called the **exocœle.**

The amnion and chorion arise together from the so-called **amniotic folds** in the sauropsida (Fig. 65). In the mammals the chorion is derived from the trophoderm, while the amnion is formed either by amniotic folds or vesicles.

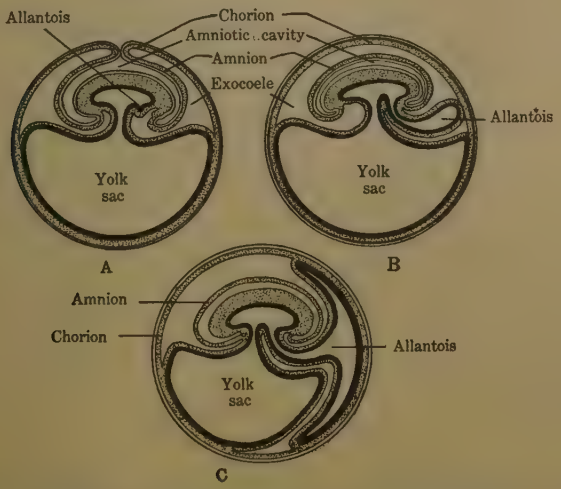

FIG. 65. — Diagrams of extra-embryonic membranes. A, amnion and chorion arising by folds. B, allantois arising from hind-gut. C, allantois and yolk sac in contact with chorion. (After Kerr.)

The chick. — In the chick, the amnion is a thin sac of somatopleure completely surrounding the embryo and continuous with the somatopleure of the embryo at the umbilicus. It contains the amniotic cavity, filled with amniotic fluid. The chorion is a similar sac pressed up against the shell membrane and connected with the amnion by a plate known as the sero-amniotic raphe.

The process of amnion and chorion formation begins in the region anterior to the head fold of the embryo, known as the **proamnion** (Fig. 156), which at first contains no mesoderm.

Here the ectoderm shows a thickening, which gradually extends around the head on either side. This thickening indicates the line of separation between the amnion-forming and the chorion-forming somatopleure. The cœlom on either side of the

head expands to form the **amnio-cardiac vesicles** (Fig. 156), elevating the somatopleure which fuses and slips over the head of the embryo, assisted by a downward bending of the head known as the cephalic flexure. This forms the **head fold of the amnion.** The lateral extension of this fold causes the elevation and fusion of the **lateral folds.** The **tail fold of the amnion** arises independently in the same manner as the head fold but entirely from somatopleure, as there is no area free from mesoderm comparable to the proamnion.

The cavity of the amnion is formed by the fusion of the amniotic folds. Soon after its completion, muscle fibers arise in the amniotic wall and initiate a rhythmic contraction which

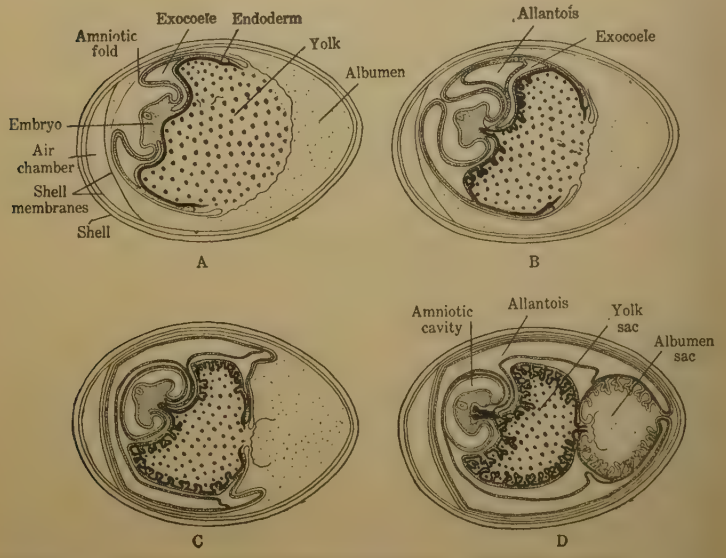

FIG. 66. — Diagrams of adnexogenesis in the chick. Transverse sections. A, amniotic folds. B, early allantois. C, allantois replacing exocœle. D, allantois forming albumen sac. (After Duval.)

rocks the embryo in the amniotic fluid and prevents adhesion to the amniotic wall. The cavity of the chorion (exocœle) is continuous with the cœlom as it is the space between the somatic and splanchnic mesoderm. As the splanchnopleure grows down

over the yolk, the chorion-forming somatopleure accompanies it until the exocœle forms a great cavity separating the embryo, amnion, and yolk sac from the shell membrane against which the chorion presses. This cavity is interrupted only by the sero-amniotic raphe, where continuity with the amnion is preserved. Later, the exocœle is obliterated by the ingrowth of yet another embryonic membrane, the allantois (Fig. 66).

Meantime the grooves at the junction of the embryo and amnion (**limiting sulci**) are invaginating beneath the embryonic body and approaching each other. The result is that they crowd against the yolk stalk and surround it as a short, hollow tube, which is called the **umbilical stalk.** The space between the outer wall of somatopleure and the inner wall of splanchnopleure is continuous with the embryonic cœlom above and the exocœle below. After the resorption of the yolk sac just before hatching, the umbilicus contracts, and its connection with the embryo is lost at hatching.

Mammals. — In the placental mammals, the formation of amnion and chorion is complicated by the early separation of

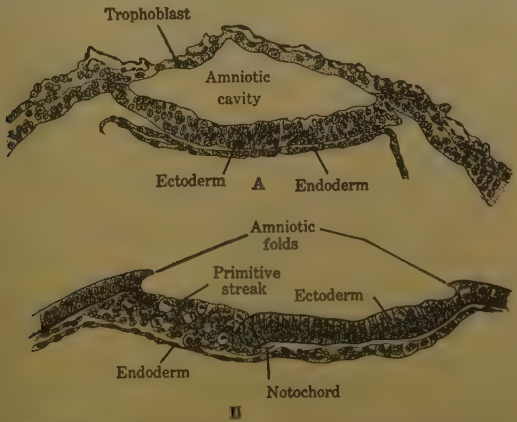

Fig. 67. — Amnion formation in the bat. A, primary amniotic cavity. B, origin of amniotic folds. (After Van Beneden.)

the trophoblast from the embryonic knob. The trophoblast, which later forms the ectoderm of the chorion, is developed precociously, consistently with its function of aiding the **implanta-**

tion of the embryo in the uterine wall. This is accomplished by the formation of enzymes which enable the embryo to penetrate into the wall of the uterus which has been prepared for this implantation during the **œstrum** (see page 147).

Type A. — There are two major types of amnion formation. In the first type, as observed in the rabbit, the ectoderm of the embryonic knob, together with the endoderm (Fig. 67), flattens to form the embryonic shield which, as has been said, is homol-

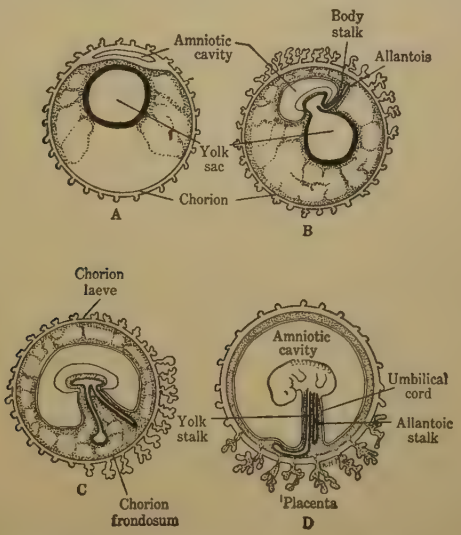

Fig. 68. — Diagrams of adnexogenesis in man. Four stages. (After Corning.)

ogous with the area pellucida of the chick. The trophoblast overlying the embryonic plate disintegrates, the several cells persisting for a longer or shorter period as **Rauber's cells.** The amnion and chorion are formed by amniotic folds, of which the internal limb of extra-embryonic somatopleure later forms the amnion, as in the chick. The outer limb of each fold, however, is formed of trophoblastic ectoderm associated with somatic mesoderm, and gives rise to the chorion. The exocœle gradually makes its way around the yolk sac by the extension of the mesoderm which follows the endoderm and delaminates into somatic and splanchnic layers.

Type B. — In the second type of amnion formation, as exemplified in the guinea pig and man (Fig. 68), the embryonic knob does not flatten out to form an embryonic shield, but the amnion arises as a vesicle in the dorsal half of the embryonic knob, just as the yolk sac of man arises as a vesicle in the ventral half. This vesicle enlarges and the floor becomes the ectodermal part of an embryonic shield as the roof of the yolk sac becomes the endodermal part. The mesoderm growing out from the primitive streak delaminates into the somatic and splanchnic layers which grow around the amnion, yolk sac, and chorion. In this type the trophoblast remains intact and becomes the ectoderm of the chorion.

The primitive type. — There has been some controversy concerning the relative primitiveness of the two methods of amnion formation just described. Apparently the method of fold formation is more nearly like the condition found in reptiles, and the amnion-forming somatopleure is the same in both cases, while the trophoblast might represent the chorion-forming ectoderm. An objection to this theory has been raised because of the fact that Rauber's cells represent the original continuous trophoblast. Accordingly, it may be supposed that the trophoblast represents the entire ectoderm of the chorion, formed precociously, and that the amniotic and embryonic ectoderm arise as a vesicle in the embryonic knob which is comparable to the entire blastoderm of the reptile less the chorionic ectoderm. The later type, in which the ectoderm flattens and amniotic folds appear, represents a reversion to the reptilian condition correlated with an advance in the formation of the embryonic shield.

In all the mammals, the amnion and chorion retain or acquire, according to the method of amnion formation, a sero-amniotic raphe; and the limiting sulci cut off the embryo from the trophoblast except at the umbilicus, which is often prolonged as the umbilical cord, containing the yolk stalk as well as the stalk of the allantois.

C. THE ALLANTOIS AND THE PLACENTA

The allantois. — With the interposition of the amniotic and chorionic walls between the developing embryo and the external world, new problems of metabolic exchange arise, with regard

particularly to the supply of oxygen, and the disposal of carbon dioxide as well as of nitrogenous waste. This exchange is carried on by means of a thin-walled sac, the **allantois,** which arises as an outgrowth of the hind-gut, and comes to occupy the space between the amnion and chorion, thus replacing the exocœle by an **allantoic cavity** (Fig. 65). The walls of the allantois are lined with capillaries which form a second extra-embryonic vascular area, the **allantoic circulation.** It functions, therefore, as an extra-embryonic bladder and an extra-embryonic respiratory organ. The allantois, like the amnion and chorion, is found only in the embryos of reptiles, birds, and mammals. In the more primitive of these, the sauropsida, and the egg-laying mammals, it is the only extra-embryonic respiratory organ.

The chick. — The allantois of the chick arises as a ventral evagination of the hind-gut soon after the formation of the tail fold (Fig. 66*B*). This diverticulum grows down into the cœlom where the distal end enlarges until it fills the exocœle, while the proximal end is caught up by the developing umbilicus, within which it forms the **allantoic stalk** (Fig. 66*C*). The wall of the allantois, like that of the enteric cavity, is formed of splanchnopleure. The outer wall fuses with the inner wall of the chorion to enclose the amnion and yolk sac, and to form about the albumen a sac-like structure to be known hereafter as the **albumen sac** (Fig. 66*D*). The sero-amniotic raphe becomes perforated, thus establishing communication between the amniotic cavity and the albumen sac. This sac also opens into the yolk sac by way of a small opening, the **yolk-sac umbilicus,** where the walls of splanchnopleure which formed the latter structure failed to meet. When the chick hatches, the yolk sac and albumen sac are emptied of their contents, shrivel up, and are resorbed into the cœlom by way of the umbilicus. Amnion, chorion, and allantois likewise shrivel up and are left with the fragments of shell and the shell membranes.

The mammals. — All mammals form an allantois, which originates like that of the chick, but whose later history varies in different groups. In the egg-laying monotremes, this history is typically sauropsidan. Among the marsupials and placental mammals, with the exception of the primates, it is usually a large structure, replacing the exocœle and contributing to the

structure of a **placenta** of some type. In the primates, including man, it is a vestigial structure.

The placenta. — This peculiarly mammalian organ is the structure by which the embryo is in contact with the uterine mucosa and by means of which the fœtus obtains nutriment from the maternal blood supply. The primary origin of the placenta is from the trophoblast, but it also receives extra-embryonic mesoderm. Its blood vessels are typically those of the allantoic circulation. Two types of placentation are distinguished according to the degree of union between the trophoblast and the lining of the uterus (**mucosa**), and a second method of distinction is made according to whether the wall of the allantois comes in contact with the chorion or not.

Indeciduate type. — The first type of placenta is called **indeciduate.** In this type, found in the marsupial mammals and the several placental groups, particularly the ungulates, the

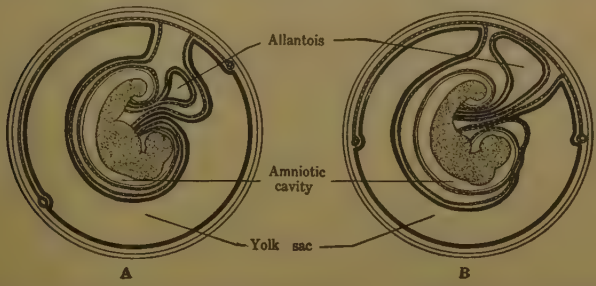

FIG. 69. — Diagrams of marsupial "placentæ." A, Didelphys. B, Perameles. (After Jenkinson.)

trophoblast is closely applied to the mucosa but both retain their integrity. The blood vessels of the placenta absorb food material excreted by the mucosa and exchange carbon dioxide for oxygen by diffusion.

Marsupials. — Among the marsupials are found both non-allantoic and allantoic "placentæ." In the opossum, Didelphys (Fig. 69*A*), the enlarged yolk sac is pressed against the trophoblast, which in turn is closely applied to the mucosa, forming folds which project into depressions in the uterine wall. The absorbed nutriment is conveyed to the embryo by means of the

area vasculosa of the yolk sac. In Perameles (Fig. 69*B*), an allantoic "placenta" is formed by the union of the allantoic sac with the trophoblast. Where this "placenta" touches the mucosa, the epithelium of the latter thickens and is invaded by maternal capillaries. The trophoblast is said to be resorbed so that the capillaries of the allantois come into intimate connection with those of the uterus. It should be mentioned in this connection that Perameles also possesses a well-developed area vasculosa on the yolk sac, and that, therefore, it is very probable that both yolk sac and allantoic circulations are concerned with the nutrition of the developing young.

Ungulates. — In the ungulates there is a well-developed allantoic placenta of the indeciduate type (Fig. 70). The blastocyst

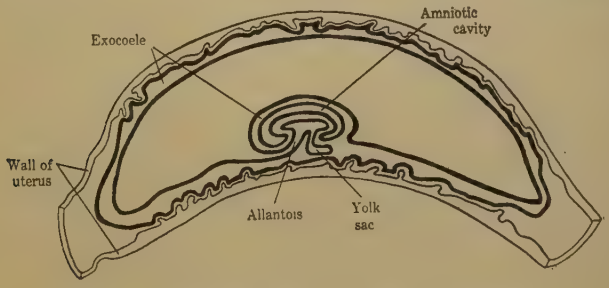

FIG. 70. — Diagram of placenta of pig. (After Smith.)

elongates, and over its surface appear projections of the trophoblast containing each a core of mesoderm. These projections, **villi**, grow into corresponding depressions of the uterine wall, called **crypts**. The allantois meantime has filled the exocœle and capillaries from the allantoic arteries and veins penetrate the mesodermal cores of the villi. These capillaries are brought very near those of the uterine wall, but the blood remains separated from that of the mother by (1) the endothelial lining of the maternal capillaries, (2) the connective tissue of the mucosa, (3) the epithelium of the mucosa, (4) the trophoblast, (5) the mesoderm of the villi, and (6) the endothelial lining of the fœtal capillaries (Fig. 73*A*, *B*). At birth the villi are pulled out of the crypts, and the placenta, with the remaining embryonic membranes, is discharged as the **"after-birth."**

Deciduate type. — The second type of placentation is called **deciduate.** In this type the trophoblast attacks the mucosa and erodes part of the lining. It is characteristic of the majority of the unguiculates (clawed mammals) and primates. In the first group the placenta is allantoic; in the primates, non-allantoic.

Carnivores. — In the carnivores (Fig. 71), is found a deciduate placenta of the allantoic type. The blastocyst elongates although not to the extent that it does in the ungulates. During

FIG. 71. — Diagram of placenta of dog. (After Jenkinson.)

the œstrum (page 147) the epithelium of the uterus is cast off. At the circular zone of the uterus, which is in contact with the equator of the blastocyst, the epithelium of the mucosa fails to regenerate. Into this zonary area grow the villi of the trophoblast, which become penetrated by the allantoic capillaries. The villi send out branched processes, each with its capillaries, which surround the maternal capillaries. Thus the maternal blood is separated from that of the fœtus by (1) the endothelium of the maternal capillaries, (2) a varying amount of maternal connective tissue, (3) the trophoblast, (4) a varying amount of chorionic connective tissue, and (5) the endothelial lining of the fœtal capillaries (Fig. 73*C*). At birth a certain amount of maternal tissue is torn away with the placenta.

Man. — In the human placenta (Fig. 72), there is the most intimate contact between the maternal and fœtal circulation as well as a non-allantoic placenta. It will be recalled that the ectoderm (and amniotic cavity) and the endoderm (and yolk sac) originated as vesicles in the embryonic knob, which retains its connection with the trophoblast as the **body stalk.** The tissue that composes this stalk may be considered as precociously

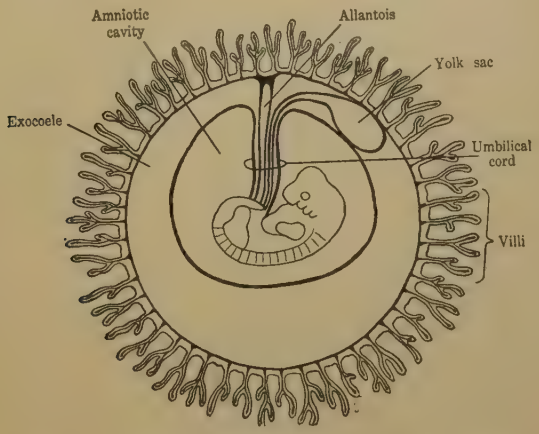

FIG. 72. — Diagram of placenta (human). (After Kölliker.)

developed allantoic mesoderm. Into the stalk grows a small endodermal evagination from the hind-gut which is the endodermal part of the allantois. It never comes in contact with the trophoblast, and soon degenerates. The limiting sulci of the amnion come together as the walls of the umbilical cord. This encloses, therefore, the body stalk, yolk stalk, and allantoic stalk, a small amount of exocœle, as well as the two umbilical arteries and two umbilical veins which grow out from the body of the embryo toward the trophoblast. These umbilical blood vessels represent the allantoic vessels of all other amniotes. Later, the umbilical veins fuse and all this tissue assumes a common connective-tissue type with the exception of the blood vessels.

The chorion. — The trophoblast, while entering the uterine wall, becomes differentiated into an outer syncytial layer and an inner cellular layer. During the process of implantation, nutri-

tion is obtained by the syncytial layer, which sends out projections, or **false villi,** into the maternal tissue. Thereafter, mesodermal cores grow into the false villi, converting them into the **true villi** which later receive capillaries from the umbilical blood vessels. Some of these bore into the spongy layer as **fixation villi.** The others, losing their syncytial layer, remain in the

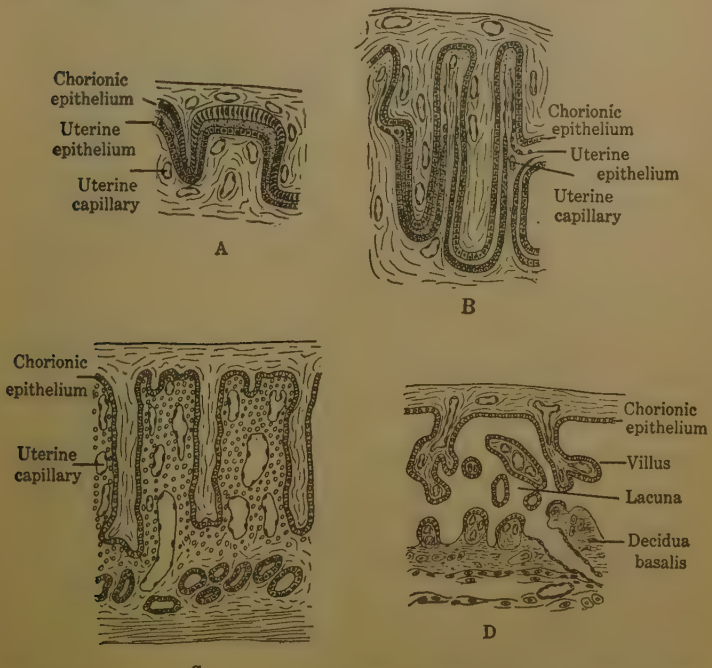

FIG. 73. — Sections of placentæ. Semi-diagrammatic. A, pig. B, cow. C, cat. D, human. (After Grosser.)

space between the trophoblast of the chorion and the maternal tissue as **nutrition villi,** bathed in maternal blood which is brought into the intervillous space and carried thence by the eroded uterine capillaries. Only those villi that are in contact with the decidua basalis persist, while the others degenerate, thus differentiating the chorion into the **chorion frondosum,** with villi, and the **chorion læve,** devoid of the same. From this account it

will appear that the maternal blood is separated from the fœtal blood stream by only (1) the cellular layer of the trophoblast, (2) the chorionic connective tissue of the villi, and (3) the endothelia of the fœtal capillaries (Fig. 73*D*).

The deciduæ. — It will be remembered that the blastocyst burrows into the uterine wall, eroding epithelium, connective tissue, and blood vessels. As the embryo increases in size, this erosion continues and the embryo sinks into the compact layer of the mucosa and comes in contact with the spongy layer. The

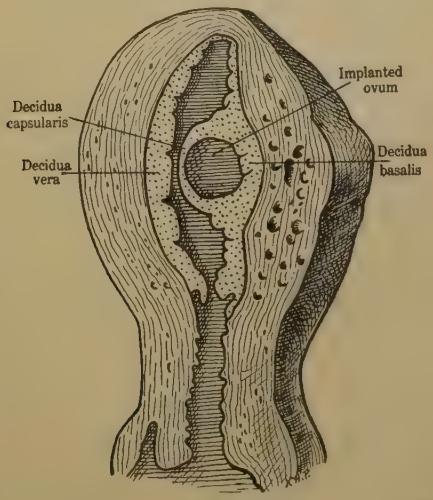

FIG. 74. — Diagram of deciduæ (human). (After Kollmann.)

mucosa grows around the burrowing embryo, shutting it off from the cavity of the uterus. There may now be distinguished three regions in the mucosa: (1) the **decidua basalis,** to which the blastocyst is attached; (2) the **decidual capsularis,** which cuts off the blastocyst from the uterine cavity; and (3) the **decidua vera,** including the remainder of the uterine lining (Fig. 74).

Parturition. — The history of the embryonic membranes, as well as of the decidua, is terminated by the birth of the fœtus, **parturition.** Owing to the absence of an allantoic sac, the amnion enlarges to fill the exocœle. Later, growth of the fœtus results in pressing the chorion læve and decidua capsularis against

the decidua vera, and obliterating the uterine cavity (Fig. 75). At birth, the placenta, carrying with it the decidua basalis, and the attached membrane, which represents the fused amnion,

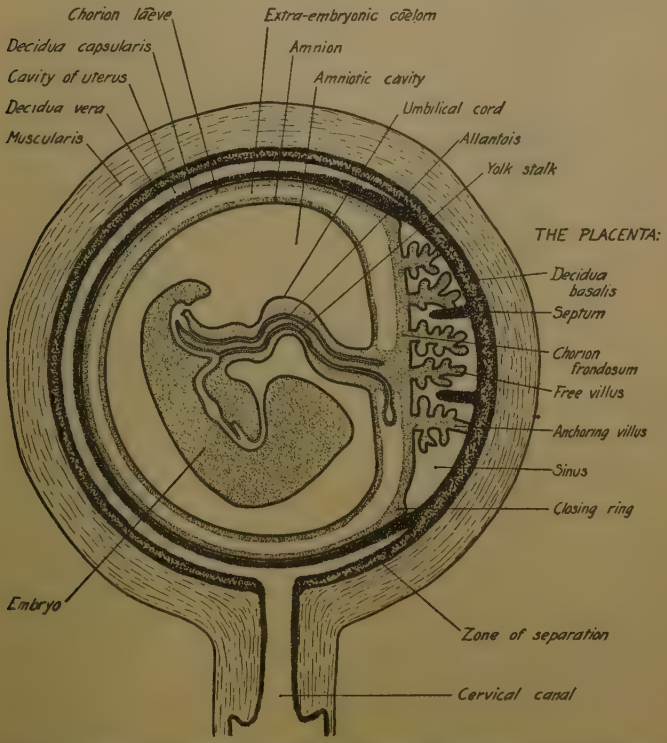

FIG. 75. — Diagram of section through the uterus at pregnancy. From Dodds, "Essentials of Human Embryology."

chorion læve, decidua capsularis, and decidua vera, are cast off as the after-birth.

D. ORIGIN OF THE EMBRYONIC MEMBRANES

There has been much speculation concerning the origin of the embryonic membranes. The yolk sac is common to all vertebrates as well as many invertebrates with lecithal eggs. It seems to have originated as an evagination of the gut resulting from the

weight of the contained yolk, as seen in the amphibia. In all telolecithal types, the formation of the embryo and that of the yolk sac occur as separate although associated events. In the anamniota, the yolk sac develops all three germ layers, in the large-yolked types as well as in the small. But, with the development of the amnion and chorion, the yolk sac is separated from the ectoderm. The endoderm of the yolk sac is a nutritive layer actively digesting the contained food; the splanchnic mesoderm gives rise to the first extra-embryonic circulation by which the food digested in the endoderm is transported to the embryonic body. The ectoderm, prior to the formation of the gills, presumably is one of the centers of respiratory exchange. It is especially significant that, in viviparous anamniotes, the vitelline circulation is the medium by which the uterine milk is conveyed into the embryonic body. It is also significant that in the monotreme mammals the yolk sac is a food reservoir, as in reptiles, and that in the marsupial mammals it sometimes takes on placental functions, while in the placentals it often becomes rudimentary.

The amnion and chorion arise as folds of the blastoderm, possibly in connection with the great size of the yolk sac and the cervical flexure which presses the head against the yielding yolk mass, rather than against the resistant shell. Graham-Kerr suggests that this pressure is the primary cause of the failure of the mesoderm to enter the proamnion. The early development of the head is responsible for the earlier appearance of the head fold of the amnion. In mammals the small size of the yolk sac results in the precocious development of the amniotic vesicle, and later the enlargement of the embryonic shield brings about the secondary acquisition of the amniotic folds, the tail fold appearing first this time.

The amnion and chorion protect the embryo from the external world, but in so doing they also enclose the excretion products of metabolism extracted by the embryonic kidneys. These wastes are normally accumulated in the bladder which, under amniote conditions, enlarges and projects into the exocœle. The walls of the allantois, formed in this way, are richly vascular, like the gut, and as the exocœle becomes filled by the allantois the blood vessels of the latter structure are brought in juxtaposition

with the thin chorionic wall and supply a new medium of respiratory exchange. In some viviparous reptiles the tertiary egg membranes fail to develop, and the outer layer of the " egg " is the chorion. Within are seen the highly vascular allantois and yolk sac, both of which develop " placental " functions and absorb a uterine milk secreted by the uterine walls and, presumably, act as organs of respiratory interchange with the maternal capillaries which lie close to the surface of the mucosa.

So, too, in the marsupial mammals is found either primitive yolk-sac " placentation," allantoic " placentation," or a mixture of both types at the same time. With most placental mammals, the allantois is the principal source of the fœtal placenta, combining with the trophoblast (chorionic ectoderm) and becoming ever more intimately connected with the mucous layer of the uterus. Finally, the human type is the most highly evolved placenta, formed exclusively from the trophoblast with the exception of the allantoic blood vessels, and affording the most intimate relationship between the two blood streams.

Summary. — Morphogenesis, in which the body assumes its form, is closely connected with adnexogenesis, in which the extra-embryonic membranes of the yolk sac, amnion, chorion, and allantois are formed, and both are profoundly influenced by the amount and distribution of the yolk. Growth in length is due to the combined effects of notogenesis and concrescence, of which the former seems more important in small-yolked forms, the latter in types with large amounts of yolk. Accompanying growth in length is the phenomenon of metamerism, or serial repetition of parts, noted primarily in the segmentation of the mesoderm. At the anterior end of the embryo, metamerism is absent or obscured by the process of cephalization, or head formation. The paired limbs arise as buds. The yolk sac is part of the body in small-yolked forms, but is an extra-embryonic appendage in types with much yolk, and is connected by a yolk sac. It arises from splanchnopleure and is richly vascular. The amnion and chorion typically arise from folds which fuse over the embryo and produce an inner membrane, the amnion, and an outer one, the chorion. Both are derived from somatopleure and are not primitively vascular. The allantois arises as an evagination from the hind-gut and typically expands to occupy the space between the amnion and chorion. It arises from splanchnopleure and, like the yolk sac, is highly vascular. In mammals, with the prolongation of intra-uterine fœtal life, either the yolk sac, in some few

forms, or the allantois, in the great majority, comes into intimate relationship with the chorion (derived from trophoblast in this case) and gives rise to the placenta, an organ of interchange between the maternal and fœtal blood streams, connected with the embryo by the umbilical cord. In the primates, neither yolk sac nor allantois enters into the composition of the placenta, but the allantoic blood vessels grow out into the chorion to supply the placental circulation. In some mammals, such as the carnivores and primates, part of the uterine wall is cast off with the placenta at parturition, a phenomenon characteristic of deciduate placentation.

REFERENCES

Assheton, R. 1916. Growth in Length.
Brachet, A. 1921. Traité d'Embryologie, Book 4, Chap. 4; Book 5, Chap. 3.
Boyden, E. A. 1918. Vestigial Gill Filaments in Chick Embryos, etc., Am. Jour. Anat., Vol. XXIII, 215–235.
Dodds, G. S. 1929. The Essentials of Human Embryology.
Graham-Kerr, J. 1919. Textbook of Embryology, Vol. II, Chaps. 7 and 8.
Hertwig, O. 1906. Handbuch, Chaps. 6, 7 and 8.
Jenkinson, J. W. 1913. Vertebrate Embryology, Chaps. 8 and 9.
Lillie, F. R. 1916. The Development of the Chick, Rev. Ed., Chap. 7.
Marshall, F. H. A. 1922. The Physiology of Reproduction, 2nd Ed.

PART II

ORGANOGENY

CHAPTER V

ENDODERMAL DERIVATIVES

The tissues derived directly from the endoderm are for the most part of the epithelial type and form the inner lining of the **enteric cavity** and the organs that arise therefrom. These organs are grouped into two closely connected organ systems, the **digestive system** and the **respiratory system.** The alimentary tube, however, becomes ensheathed in splanchnic mesoderm which contributes largely to the ultimate structure of the organ systems just mentioned. Furthermore, this tube opens to the exterior at both the cephalic and caudal ends by means of two ectodermal pits, the **stomodæum** and **proctodæum,** respectively. All three germ layers, therefore, contribute to the organogeny of these systems.

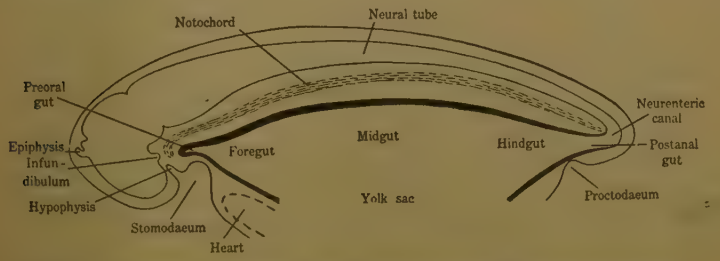

FIG. 76. — Diagram of an early embryo, sagittal section, to show endodermal derivatives.

The stomodæum. — There is an ectodermal invagination on the ventral side of the head to form the stomodæum (Fig. 76), which is bounded on the sides by the **maxillary ridges** and on the rear by the **mandibular ridges.** The rupture of the oral plate,

which separates the stomodæum from the fore-gut, results in the formation of the **oral cavity,** or **mouth.** From the stomodæum another invagination, the **hypophysis,** grows upward in front of the fore-gut, and eventually fuses with an evagination from the floor of the neural tube, the **infundibulum,** to form the **pituitary gland,** an organ of internal secretion. As the stomodæum joins the fore-gut a little posterior to the cephalic end of the latter cavity, there is a blind pocket of endoderm, anterior to the mouth, called the **preoral gut.**

The oral cavity. — The cavity of the mouth is a compound structure, derived in part from the ectodermal stomodæum and in part from the endodermal fore-gut. The boundary line between these is soon lost after the rupture of the oral plate owing to unequal local growth of the different regions of the mouth. The boundaries of the mouth are the **upper jaws,** formed from the

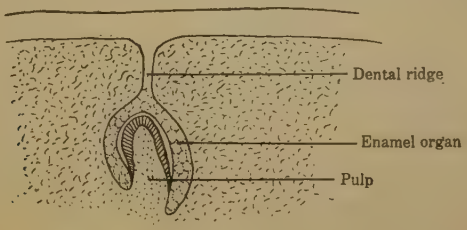

FIG. 77. — Diagram of developing tooth.

maxillary ridges, and the lower jaws, derived from the **mandibular ridges.** On these ridges the **teeth** arise in exactly the same way as the placoid scales of the elasmobranchs. Two elements are concerned: an ectodermal **enamel organ,** shaped like an inverted cup; and a mesodermal **dental papilla,** which fills the cavity of the enamel organ. The enamel organ gives rise to the outer enamel layer of the tooth, while the papilla forms the dentine (Fig. 77). The dentine is in the general form of a hollow cone, the cavity of which is filled with connective tissue, nerves, and blood vessels. The **tongue** (Fig. 78) is also a compound organ, arising from an endodermal **primary tongue** which is formed from the floor of the pharynx in the region of the hyoid arches, and from an ectodermal **secondary tongue** which arises from the floor of the oral cavity in front of the thyreoid gland (page 122).

Into the tongue a migration of mesoderm takes place, by means of which the musculature is formed. The glands of the mouth (**salivary glands,** etc.) arise from the ectodermal lining of the mouth. The connection between the oral cavity and the nasal cavity will be discussed in Chapter VII.

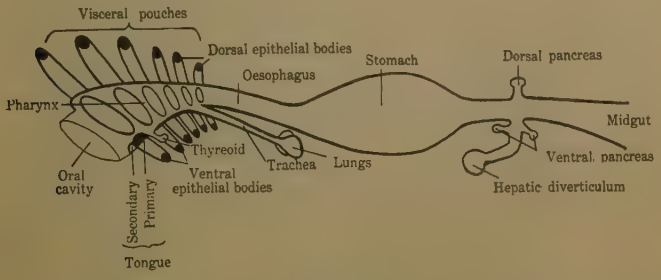

FIG. 78. — Diagram of the derivatives of the fore-gut.

The pharynx. — The region of the fore-gut which follows the oral cavity is the **pharynx,** particularly important on account of the respiratory organs and other structures which arise from it.

Respiratory organs. — Respiratory exchange may take place in any thin epithelium in which the blood corpuscles are brought into contact with the oxygen-carrying medium. These epithelia may be either ectodermal or endodermal in origin. Thus, we find that among the amphibia, respiration may take place in the skin as a whole (lungless salamanders); in specialized outgrowths on the visceral arches, **external gills;** or in the so-called " hairs " of the African frog, Astylosternus. In this group are to be found also examples of endodermal respiratory organs, the **internal gills** and **lungs.** Internal gills are otherwise found only among the aquatic classes, the pisces, while the lungs are characteristic respiratory organs of land forms, the amniota. Both internal gills and lungs arise from the pharynx.

The internal gills. — The internal gills, **branchiæ,** arise in the visceral clefts (Fig. 78) common to all chordates. Among the vertebrates these are typically six in number, consisting of a **spiracle** and five **branchial clefts,** separated by the visceral arches, of which the first, anterior to the spiracle, is known as the **mandibular arch;** and the second, posterior to the spiracle, is

called the **hyoid arch.** The visceral clefts are formed by the coming together of paired evaginations of the endoderm (visceral pouches) and complementary invaginations of the ectoderm (visceral grooves). The ectoderm and endoderm come into direct connection to form **closing plates.** Later, these plates rupture and a series of finger-like projections grow out into the cleft from the anterior and posterior sides of each arch. These filamentous processes usually fuse to form a **demibranch** (Fig. 153). In some fish the demibranchs are apparently of endodermal origin, while in the amphibia they are derived from the ectoderm. In the spiracle a gill-like structure, the **pseudobranch,** develops among the fish. In higher forms the spiracle does not open to the exterior but gives rise to the **tympanic cavity** and **auditory tube** (see Chapter VII). In all fish except the elasmobranchs, a projection grows back from the hyoid arch to cover the remaining visceral clefts. This is the **operculum.** Internal gills do not appear in the development of the amniota; but the visceral clefts, or at least the visceral pouches and grooves, are of invariable occurrence.

The lungs. — In all of the vertebrates except the cyclostomes and chondrichthyes, there develops from the pharynx a sac (or pair of sacs) which becomes the **air bladder** in pisces and the **lungs** in tetrapoda. We shall confine our attention here to the development of the lungs (Fig. 78). The first indication of lung formation is the appearance of a longitudinal groove in the floor of the pharynx posterior to the last pair of visceral pouches. This is the **tracheal groove.** This groove separates from the pharynx, the process commencing at the posterior end, so that the dorsal portion of the tube, or **œsophagus,** is separated from the ventral portion, or **trachea,** except for a narrow opening, the **glottis.** The trachea grows backward rapidly and divides into two lobes, the primordia of the lungs. In the birds and mammals these lung primordia subdivide many times to form the **bronchi,** or branches of the respiratory tree.

The thyreoid gland. — This structure arises as a median ventral evagination of the pharyngeal floor between the primary and the secondary tongue primordia or at the level of the hyoid arches. The diverticulum grows downward and expands at its distal end (Fig. 78). Eventually, its connection with the pharyngeal

floor, the **thyreoglossal duct,** becomes occluded and disappears, and the gland itself subdivides into a mass of vesicles which migrate backward and assume somewhat different positions in various vertebrates, often ending as a paired organ on either side of the trachea.

The epithelial bodies. — In all the vertebrates there arise, from the upper or lower angles of the visceral pouches, small buds of epithelium which often give rise to endocrine glands of varying — and mostly unknown — function (Figs. 78, 79). The dorsal buds (except among the mammals, where conditions are reversed) contribute in varying number to the formation of a

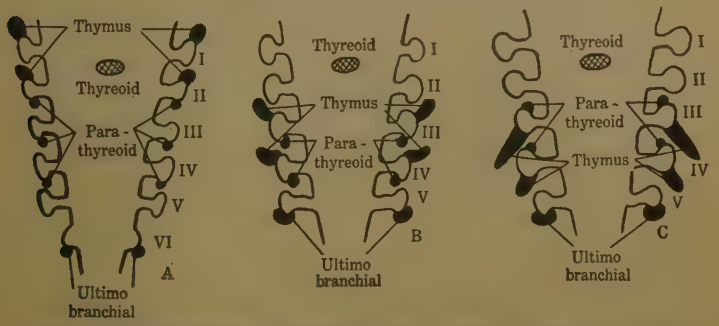

FIG. 79. — Diagram of the epithelial bodies. A, frog. B, chick. C, man.

large gland, the **thymus,** which loses connection with the pharynx and moves backward to its definitive position, which differs according to the form studied. The remainder of the dorsal bodies become lymphoid and degenerate. The ventral buds (absent in fish) detach themselves from the pharyngeal wall and take up varying positions. Among the mammals it is the ventral buds which form the thymus, while the dorsal buds of the third and fourth pouches move to the sides of the thyreoid gland where they are known as the **parathyreoids.**

The œsophagus. — The alimentary canal behind the pharynx becomes specialized into four regions: (1) the **œsophagus;** (2) the **stomach;** (3) the **intestine** and its derivatives; and (4) the **cloaca.** Of these, the œsophagus (Fig. 78) remains comparatively unspecialized; it is a narrow tube, short in the anamniota, elongate in the amniota. No digestive glands are found in this region.

The stomach. — This portion of the alimentary tract is distinguished by its dilation (Fig. 78) into a large sac or series of sacs, and by the development of a thick wall of muscle from the splanchnic mesoderm in which it is enveloped. The stomach is rich in glands which aid in digesting the passing food.

The intestine. — All of the regions of the alimentary tract mentioned so far are derived from the fore-gut. The intestine is derived in part from the fore-gut, in part from the mid-gut, and in part from the hind-gut. It is impossible to indicate exactly which regions arise from these divisions of the gut, as both the fore-gut and the hind-gut expand at the expense of the mid-gut during the consumption of the yolk. As was said in the discussion of the development of body form, the division of the alimentary canal into these regions is the result of the method by which the head and tail are formed. The intestine becomes subdivided in various ways in the different groups, but we need notice only the most anterior of these, the **duodenum,** which is that portion of the intestine immediately succeeding the stomach and generally held to be derived from the fore-gut. The intestine is richly glandular throughout its length, but from the duodenum, in particular, we find developed two most important glands, the **liver** and the **pancreas** (Fig. 78).

The liver. — This gland arises from the ventral side of the duodenum as an evagination which grows forward, expanding into a vesicle at the distal end and retaining its connection with the duodenum by a narrow hollow stalk, the **ductus choledochus** (Fig. 78). The sac-like distal end becomes subdivided, by the ingrowth of mesenchyme, into many tubules which often anastomose. In this process of growth and subdivision the liver grows about the vitelline veins (Chapter VI) and breaks these up into a system of **hepatic capillaries.** The cavity of the sac becomes the **gall-bladder,** to which the bile, formed in the glandular portion of the liver, is carried by means of the **hepatic ducts.** It releases these secretions into the duodenum *via* the ductus choledochus.

The pancreas. — This gland arises usually from three diverticula of the duodenum (Fig. 78), but the number of primordia is variable. One appears on the dorsal side of the duodenum just posterior to the stomach; the others arise on the ventral

side, usually in connection with the hepatic diverticulum. The primordia increase in size, and break up into masses of secretory tubules at the distal end of each. The proximal ends become the **pancreatic ducts,** one or more of which may be suppressed in later organogeny.

The cloaca. — The intestine behind the duodenum is variously subdivided in the different vertebrate classes, but all agree in the possession of a terminal region which receives in addition the ends of the nephric ducts and of the gonoducts (see Chapter VI). From the cloaca also arises the **urinary bladder** and the **allantois** of the amniotes.

The cloaca, like the pharynx, communicates with the exterior by means of an aperture lined with ectoderm, which arises as a median ventral pit, the **proctodæum** (Fig. 76), just in front of the tail region. The proctodæum is formed at the point where the blastopore was obliterated and is separated from the hind-gut temporarily by means of the **cloacal plate,** which is comparable with the oral plate. For a time there is a blind pocket of endoderm posterior to the cloaca, which is known as the postcloacal gut. The region of the cloaca anterior to the entrance of the nephric ducts is known as the **rectum.** In mammals this region becomes separated from the remainder of the cloaca, which is then known as the **urogenital sinus.** Each of these cavities has a separate exit, the two openings being the **anus** and the **urogenital aperture,** respectively.

The frog (*See also Chapter VIII*). — The mouth of the tadpole does not open until a few days after hatching. It remains round during larval life and is enclosed by the mandibular ridges. Outside these, folds of ectoderm project as the larval lips, on which horny larval teeth develop. These larval structures are lost at metamorphosis, when the definitive jaws and teeth are formed in the usual way. The tongue is compound, arising from a primary tongue and a gland field, relatively late in larval life. The hypophysis is solid (Fig. 139).

Six visceral pouches appear, of which the first never becomes perforated, its closing plate becoming the tympanum of the ear, and its cavity persisting as the tubo-tympanic cavity. Of the five remaining pouches, the second and third open to the exterior before the first and fourth, while the fifth remains vestigial.

External gills appear on the third, fourth, and fifth arches, but are resorbed later when covered by the operculum. This structure fuses with the body surface on the right side, but on the left it opens to the exterior by an opercular aperture. The internal gills appear as demibranchs commencing on the anterior side of the third arch. The first three gills, therefore, have two demibranchs, while the fourth has but one, formed from the anterior side of the sixth arch. The visceral clefts, gills, and opercular cavity are lost as separate structures by cell proliferation and reorganization just before metamorphosis. The lungs appear early in larval life as solid primordia of the pharynx. These acquire cavities prior to the formation of the tracheal groove which is relatively late in formation. The thyreoid arises, just before hatching, as a solid diverticulum of the pharynx; it soon detaches itself and divides into two bodies which later become vesicular. The two thymus glands are formed from epithelial bodies on the dorsal side of the first and second visceral pouches. Epithelial bodies arise from the ventral sides of the second visceral pouches. It has been claimed that those of the third and fourth pouches become the **carotid glands.** The sixth pharyngeal pouches give rise to the **ultimobranchial** (**suprapericardial**) **bodies.** (Fig. 79*A*.)

The œsophagus is short, and the stomach a simple dilation. The liver arises as a backward ventral diverticulum of the duodenum (Fig. 139). All three pancreatic primordia appear and fuse; the dorsal duct disappears, while the two ventral ducts fuse to become the adult pancreatic duct. The intestine of the tadpole, which is long and coiled (about nine times the body length), becomes resorbed during metamorphosis until it is about one-third of its larval length (Fig. 80).

The postcloacal gut loses its connection with the neural tube (neurenteric canal) during the backward growth of the tail. The urinary bladder does not appear until after metamorphosis.

The chick (*See also Chapter IX*). — The mouth opens on the third day of incubation. The teeth are represented only by the teeth ridges which are the first stage in the appearance of the enamel organs. These appear on the sixth day of incubation and disappear shortly after the cornification of the jaws, which results in the formation of the beak and the egg tooth, the latter

a horny projection on the upper jaw which is used in breaking through the shell at the time of hatching, and soon after disappears. The primordia of the tongue appear on the fourth day.

Five visceral pouches appear, of which the first three open to the exterior during the third day of incubation (Fig. 165). The

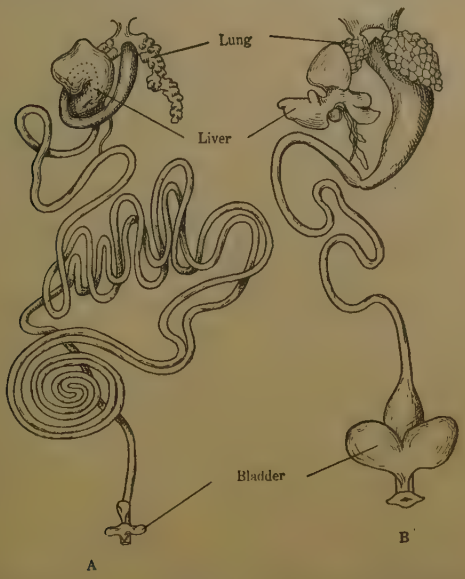

Fig. 80. — Alimentary tract. A, tadpole. B, frog. (After Leuckart wall charts.)

first cleft closes during the fourth day, and the dorsal part of the pouch becomes the tubo-tympanic cavity. With the extension of the cervical flexure, the remaining pouches are crowded together and disappear. The thyreoid appears on the second day, separates from the pharynx on the fourth, and on the seventh divides into two bodies which migrate backward to the junction of the common carotid and subclavian arteries. The thymus arises from the dorsal epithelial bodies of the third and fourth visceral pouches, while the parathyreoid rudiments arise from the ventral epithelial bodies. The fifth pouch gives rise to the ultimobranchial bodies. The lung primordia (Fig. 81) appear on the third day and grow back, becoming surrounded by mesen-

chyme. The primary bronchi subdivide to form a respiratory tree, some branches of which extend among the viscera and even into the hollow bones, as the **accessory air sacs.**

The œsophagus is relatively long, and a dilation, the **crop,** forms at its posterior end. The stomach is divided into an anterior **proventriculus,** which contains the gastric glands, and a muscular **gizzard** at the posterior end. The liver primordium arises at the edge of the anterior intestinal portal on the third day and, therefore, presents the aspect of an anterior ventral

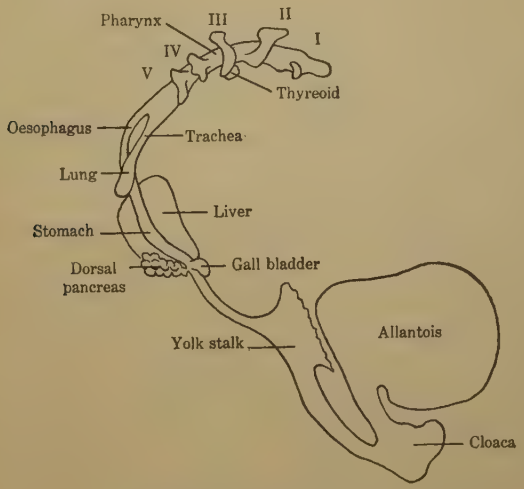

FIG. 81. — Endodermal derivatives in 72 hour chick.

and two posterior lateral diverticula for a short time. These fuse, however, by the end of that day, as the backward extension of the fore-gut continues. Three pancreatic diverticula are formed, the dorsal one on the third day, the ventral ones on the fourth. They fuse in later development, and either two or three of the ducts persist. The anterior portion of the mid-gut becomes the small intestine, the large intestine arising from the posterior region.

The cloaca is first distinguishable on the fourth day, when the proctodæum also is first apparent. The cloaca is ultimately divided into three regions: an anterior portion, the **coprodæum,**

into which the rectum enters; an intermediate part, the **urodæum,** into which the nephric ducts and gonoducts enter; and the terminal **proctodæum.**

Man (*See also Chapter X*). — The mouth opens in the second or third week, and, like that of all vertebrates, develops lips (fifth week). Ten teeth papillæ and enamel caps, the primordia of the milk teeth, appear in each jaw. This is a long-drawn-out process, the germs of the third molar not appearing until the fifth year of infancy. The tongue arises from swellings on the first three arches, the secondary tongue, or gland field, appearing as the **tuberculum impar,** which does not, however, appear to contribute to the ultimate structure of the tongue.

Five pairs of visceral pouches appear, none of which becomes perforated. The first gives rise to the tubo-tympanic cavity. The ventral portion of the second persists as the fossa in which the tonsil develops. The dorsal epithelial bodies from the third and fourth pair of pouches become the parathyreoids. The ventral epithelial bodies of the third pair of pouches unite to form the thymus gland. Similar bodies from the fourth pair may give rise to vestigial thymus-like bodies which remain attached to the parathyreoids from the same pouch. The fifth pair become the ultimobranchial bodies. The thyreoid gland undergoes an incomplete division into two lobes which remain connected by a narrow isthmus. The lungs (Fig. 82) arise toward the end of the fourth week, from a laryngo-tracheal groove. The cartilages and musculature of the larynx arise from the branchial arches.

The œsophagus, at first relatively short, lengthens as the backward movement of the heart and lungs displaces the stomach. The latter organ arises as a dilation of the fore-gut posterior to the œsophagus. Continued growth, mainly on the dorsal surface, produces the **greater curvature,** and a displacement of the whole organ so that the cephalic end is moved to the left and the caudal end to the right. This is followed by a rotation of the stomach on its long axis through ninety degrees to the left. The liver arises during the third week as a ventral groove in the duodenum. The pancreas appears slightly later, with either two or three primordia according to whether or not one of the ventral primordia be suppressed. The ventral pancreatic duct persists and opens into the ductus choledochus. The point of

division between small and large intestines is marked by the formation of a blind pouch, the **cæcum.** The distal end of the cæcum does not grow as rapidly as the proximal region and so remains a finger-like projection known as the **vermiform appendix.** The small intestine, growing more rapidly than the large,

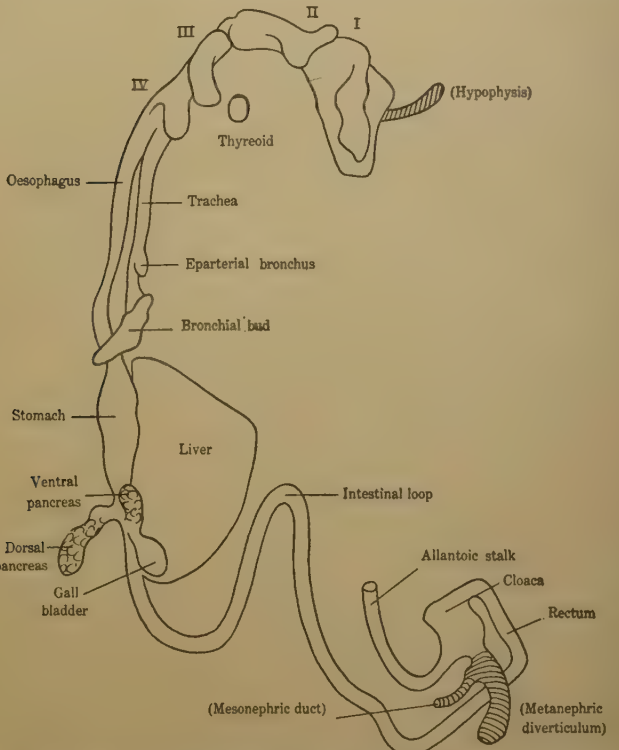

FIG. 82. — Endodermal derivatives in 10 mm. pig. (From a wax reconstruction by G. W. Hunter and L. T. Brown.)

is thrown into a set of six primary coils, each of which develops secondary coils.

The cloaca becomes divided, by a frontal partition, into a dorsal rectum and a ventral urogenital sinus. The cloacal membrane is correspondingly divided into a rectal and a uro-

genital plate, and the final openings are the anus and the urogenital aperture.

Summary. — The endoderm gives rise to the epithelial lining of the following structures:

A. Fore-gut
  I. Oral cavity (also partly from ectoderm)
    (Teeth, ectodermal)
    (Tongue, also partly from ectoderm)
    (Salivary glands, ectodermal)
  II. Pharynx
    Trachea and lungs
    Thyreoid
    Pharyngeal pouches
      Auditory tube and chamber
      Fossa of palatine tonsil
      Thymus
      Parathyreoids
      Ultimobranchial bodies
  III. Œsophagus
  IV. Stomach
  V. Duodenum
    Liver
    Pancreas
B. Mid-gut
  I. Intestine
C. Hind-gut
  I. Cloaca (also partly from ectoderm)
    Rectum (also partly from ectoderm)
    Urogenital sinus (also partly from ectoderm)
    Urinary bladder
    Urethra (also partly from mesoderm)
    Allantois

REFERENCES

Brachet, A. 1921. Traité d'Embryologie des Vertébrés, Part 2, Bk. 1, Chap. 5; Bk. 2, Chap. 4.
Graham-Kerr, J. 1919. Textbook of Embryology, Vol. II, Chap. 3.
Hertwig, O. 1906. Handbuch, Vol. 2, Chaps. 1, 2 and 4.
Keibel and Mall. 1910–1912. Human Embryology, Chap. 17.
Kellicott, W. E. 1913. Chordate Development.
Kingsley, J. S. 1926. Comparative Anatomy of Vertebrates, 3rd Ed.
Lillie, F. R. 1919. The Development of the Chick, 2nd Ed.
McMurrich, J. P. 1923. The Development of the Human Body, 7th Ed.

CHAPTER VI

MESODERMAL DERIVATIVES

The middle germ layer arises as three different aggregates of cells between the ectoderm and endoderm: the notochord; the mesoderm; and the mesenchyme. The origin of the notochord has already been described and its later history will be discussed in connection with the skeleton. Organs of mesenchymatous origin will be taken up in connection with the history of the region from which their mesenchyme originates. Of the structures derived from the mesoderm, we shall consider first those arising from the hypomere, then those whose origin is from the mesomere, and finally those derived from the axial epimere.

A. THE CŒLOM AND MESENTERIES

Cavities may appear in all three divisions of the mesoderm; if in the myotomes, they are known as **myocœles;** if in the nephrotomes, they are called **nephrocœles;** while the cavity of the lateral mesoderm is the **cœlom** (Fig. 56). In some forms the three cavities are confluent. The connection, however, is a temporary one and the myocœles soon disappear. In other forms they make a transitory appearance and are entirely disconnected with the other cavities, while in many vertebrates myocœles are never formed. The nephrocœles will be considered with the nephric organs. The cœlom in Amphioxus has a metameric origin from the ventral portions of the enterocœles, which become confluent at this point by the disappearance of the intervening anterior and posterior partitions. In vertebrates the cœlomic cavity arises from the splitting of the lateral mesoderm into a dorsal somatic and a ventral splanchnic layer. In the amniota this split continues out into the extra-embryonic mesoderm, thus giving rise to the **exocœle,** or cavity of the chorion. The cœlom does not extend anterior to the visceral arches. Transitory cavities have been found in the arches and, indeed,

in the head itself, and these have been interpreted as the remains of a cephalic cœlom. It will appear later that these are more probably the rudiments of cephalic myotomes. The cœlom does not extend into the tail.

Somatopleure and splanchnopleure. — The somatopleure has already been defined as the outer layer of the lateral mesoderm together with the ectoderm with which it becomes associated.

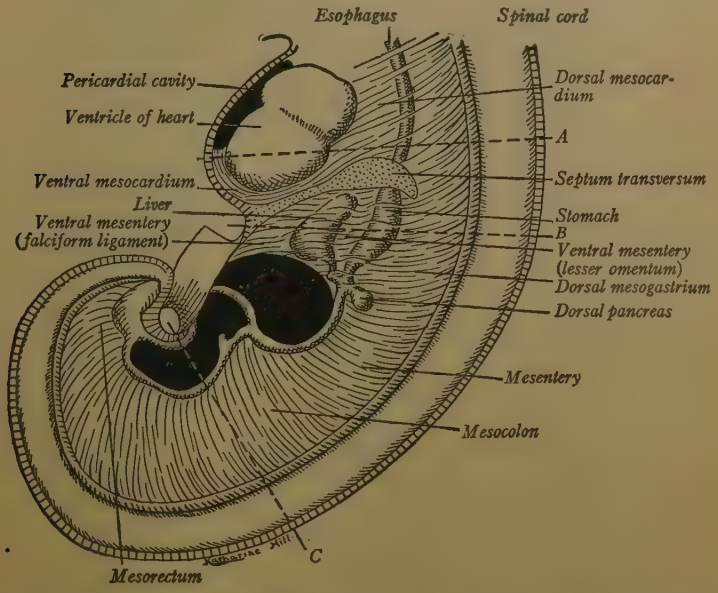

FIG. 83. — Diagram to show the mesenteries in sagittal section. A, B, and C indicate the planes of the sections in Fig. 84. (After Prentiss. From Arey, "Developmental Anatomy," copyright, 1924, by W. B. Saunders Co.)

Between these two there is an invasion of mesenchymatous cells from the dermatomes and myotomes which give rise to the **corium** of the skin (see Chapter VII) and to its **dermal musculature** (see page 177). The somatic mesoderm lining the outer wall of the cœlom becomes the **outer peritoneal lining.** The splanchnopleure is the inner layer of the lateral mesoderm plus the endoderm with which it is associated. Between these two occurs a migration of mesenchyme cells which give rise to the

splanchnic musculature and blood vessels, while the splanchnic mesoderm itself forms the **inner peritoneal lining** of the cœlom.

The mesenteries (Fig. 83). — In all the vertebrates, the cœlom is divided for a time into right and left halves by sagittal partitions above and below the alimentary canal, known as the **dorsal mesentery** and the **ventral mesentery,** respectively. These are formed by the inward growth of the splanchnic mesoderm above and below the digestive tube and the subsequent fusion of these sheets in the median line. The ventral mesentery disappears posterior to the liver, probably in connection

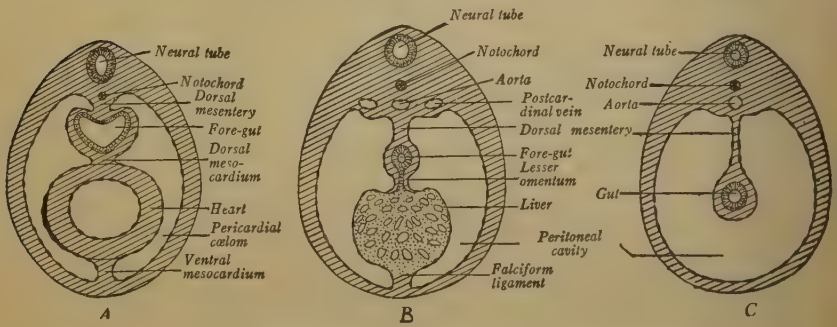

FIG. 84. — Diagrams of the mesenteries as seen in transverse sections. Compare Fig. 83. (After Prentiss. From Arey, "Developmental Anatomy," copyright, 1924, by W. B. Saunders Co.)

with the coiling of the intestine. The dorsal mesentery (Fig. 83) persists as the support of the alimentary canal, and frequently becomes subdivided into regions which are named from the supported organ, such as the **mesogaster** which supports the stomach, the **mesoduodenum,** etc. In the formation of the ventral mesentery, two organs, the heart and the liver, owing to their ventral position, are caught in between the two advancing sheets of splanchnic mesoderm. In these regions, therefore, the ventral mesentery is divided into an upper and a lower half. The ventral mesentery dorsal to the heart becomes the **dorsal mesocardium;** that part which is ventral to the heart is the **ventral mesocardium** (Fig. 84*A*). Both eventually disappear as the heart increases in size and complexity. In the region of the liver, the dorsal half of the mesentery becomes the **dorsal**

mesohepar, while the ventral portion is the **ventral mesohepar** (Fig. 84*B*). The primordia of the pancreas lie originally in the dorsal and ventral mesenteries, respectively, but with the rotation of the stomach all are included in the dorsal mesentery. The peritoneal supports of the nephric and genital organs will be considered in the following section. The **spleen** (see page 161) arises in the mesogaster, close to the wall of the alimentary canal, and is probably mesodermal in origin.

Later divisions of the cœlom. — The cœlom becomes divided into an anterior **pericardial cavity** surrounding the heart, and a posterior **abdominal cavity** surrounding the viscera, by the **septum transversum,** a transverse partition which grows out from the bridge of mesoderm surrounding the vitelline veins where they cross the cœlom *en route* from the body wall to the heart (Fig. 85*A*). These cavities are connected during a large part of the embryonic period by **pericardio-peritoneal canals**

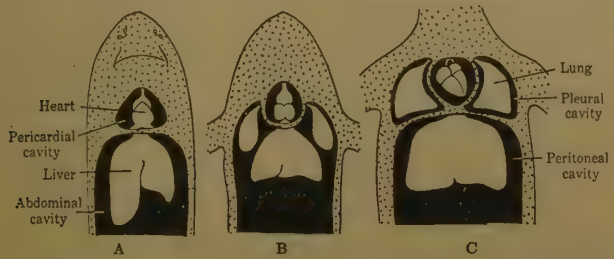

FIG. 85. — Diagram of cœlomic cavities. A, in fish. B, in amphibia and sauropsida. C, in mammals. (After Kingsley, "Comparative Anatomy of Vertebrates," copyright, 1926, by P. Blakiston's Son and Co., modified.)

where the septum has failed to unite with the ventral body wall. In the amniota, additional septa develop behind the lungs and separate the **pleural cavities,** which contain the lungs, from the remainder of the abdominal cavity, which is now known as the **peritoneal cavity** (Fig. 85*B*). The pleural cavities are separated from each other in the median line by the **mediastinum.** In the mammals (Fig. 85*C*) the partition separating the lungs from the viscera receives musculature from the myotomes and becomes the **diaphragm.**

The frog (*See also Chapter VIII*). — In the frog, the ventral mesentery disappears as soon as it has been formed, except in the

region of the heart and liver. The ventral mesocardium appears before the dorsal mesocardium is formed, and disappears soon after, to be followed by the disappearance of the dorsal mesocardium. The ventral mesohepar also has but a short period of existence. The septum transversum receives much of its substance from the mesodermal sheath of the liver. No pleural cavities are formed.

The chick (*See also Chapter IX.*) — In the chick, both dorsal and ventral mesenteries are formed. The latter, however, persists only in the region of the fore-gut, and gives rise to the mesocardia, which soon disappear; the dorsal mesohepar, which becomes the **gastro-hepatic ligament;** and the ventral mesohepar, which becomes the **ventral ligament.** The septum transversum is not completed until the eighth day of incubation. The pleural cavities are cut off from the pericardial cavities by a pleuro-pericardial septum, and from the peritoneal cavity by the pleuro-peritoneal septum.

Man (*See also Chapter X*). — The pericardial cavity is, from the first, distinguishable from the abdominal cavity, inasmuch as it never communicates directly with the extra-embryonic cœlom as does the latter cavity. As in the chick, its posterior boundary is coterminous with that of the fore-gut but it is in communication with the abdominal cavity by means of the **parietal recesses,** passages which correspond to the peritoneo-pericardial canals of the anamniotes. The recesses are divided frontally by the vitelline veins into dorsal and ventral parietal recesses. With the formation of the septum transversum, the ventral recesses are incorporated into the pericardial cavity. The dorsal recesses become the pleural cavities; and the pleuro-peritoneal septum, which divides them from the peritoneal cavity, is formed by the upward growth of the diaphragm. The musculature of this organ arises from the fourth cervical myotome during the backward growth of the diaphragm. The rotation of the stomach results in a rearrangement of the mesenteries, for an account of which the reader is referred to Hertwig or Keibel and Mall.

B. THE NEPHRIC ORGANS

The nephric or excretory system of vertebrates is essentially a paired series of tubes (**nephridia**), developed in the mesomeres, which elaborate the excretory products and discharge them to the exterior by two longitudinal **nephric ducts** which empty into the cloaca. There are three different types of **nephroi** among the vertebrates (Fig. 86). The first is the **pronephros,** which arises from the anterior nephrotomes and is the functional kidney in the larval stages of the pisces and the amphibia. The second is the **mesonephros,** which arises from nephrotomes posterior to the pronephros and is the functional kidney of adult anamniotes and embryonic or fœtal amniotes. The third is the **metanephros** which is the functional kidney of adult amniotes.

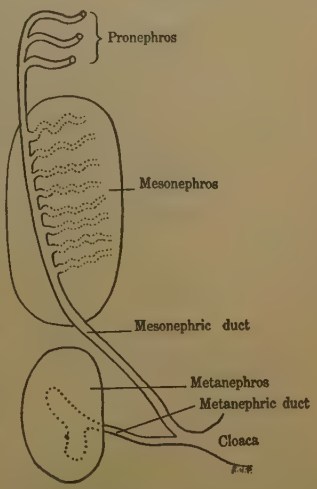

FIG. 86. — Diagram showing relationships of pro-, meso- and metanephros.

The pronephros. — This organ is formed during development by all vertebrates, but is best developed in larval types like the frog, where it arises from nephrocœles (Fig. 87) in the anterior nephrotomes (III, IV, V), the dorsal ends of which grow caudally and unite with each other to form the **pronephric duct** which grows backward toward the cloaca. The ventral ends of the nephrocœles open into the cœlom, and these openings, the **nephrostomes,** become lined with long cilia. The tubules meantime elongate and become contorted as they project into the surrounding posterior cardinal vein. Median to each nephrostome, the splanchnic mesoderm bulges out and in this projection develops a net of capillaries, or **glomerulus,** which becomes connected with the dorsal aorta. The pronephros is functional, at most, for a short time; and it disappears as the mesonephros develops to replace it.

The mesonephros. — The mesonephros, like the pronephros, is developed by all vertebrates. It becomes the adult kidney of the anamniotes, but is functional during the embryonic (and fœtal) period only of the amniotes. Portions of the mesonephros

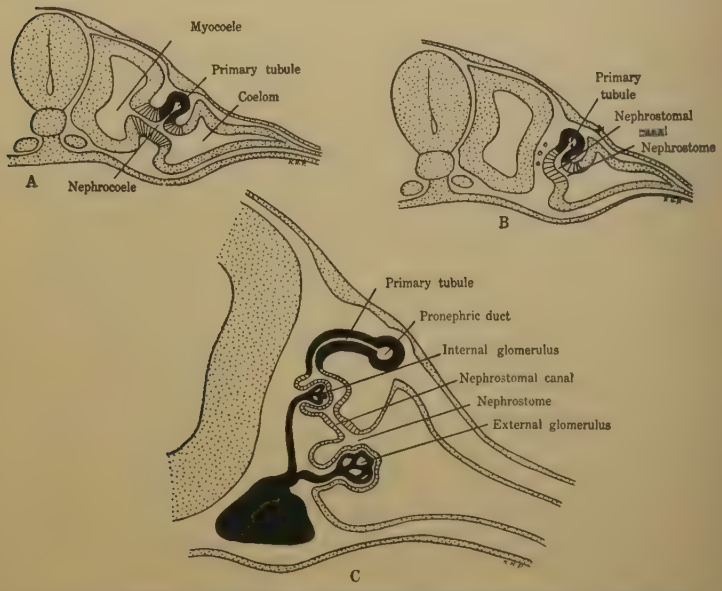

FIG. 87. — Diagrams to show development of a pronephric tubule. (After Felix.)

become associated with the genital organs of the adult (see next section).

The mesonephros also develops as a series of segmental nephrocœles, but in the nephrotomes posterior to those containing the pronephric ducts with which they unite (Fig. 88). After the degeneration of the pronephros, the tube is known as the **mesonephric** or **Wolffian duct.** The ventral ends of the nephrocœles acquire cœlomic nephrostomes in the anamniotes. In amniote development, nephrostomes are seldom formed. A glomerulus connected with the dorsal aorta and the cardinal veins arises in connection with each tubule, as in the pronephros. An important difference between the pronephros and the mesonephros lies in the fact that the number of nephric tubules in each

nephrotome is greater in the case of the mesonephros (Fig. 89). These arise by the constriction of the posterior median part of each nephrocœle into a small vesicle which gives rise to a **secondary tubule**; each of these tubules acquires a glomerulus and nephrostome at the proximal end. The connection of these

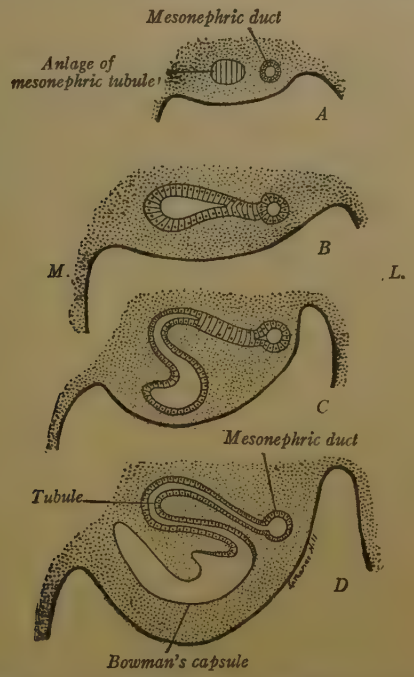

FIG. 88. — Diagrams to show development of a mesonephric tubule. (After Felix. From Arey, "Developmental Anatomy," copyright, 1924, by W. B. Saunders Co.)

secondary tubules with the Wolffian duct, however, is attained by an evagination from the duct itself which grows out as the **collecting duct** to meet the developing secondary tubule. From these secondary tubules, **tertiary** ones develop in like manner and acquire connections with the collecting duct through an evagination of this canal. As many as eight tubules may be formed in a single segment by this process of budding. This complexity is greatest at the posterior end of the mesonephros.

In the amniotes, the mesonephros (except for that portion associated with the genital organs) disappears with the development of the metanephros.

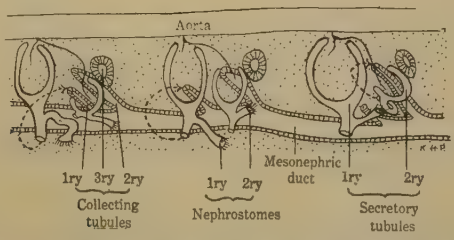

Fig. 89. — Diagrammatic reconstruction of three nephrotomes during the formation of the mesonephros. (After Brauer.)

The metanephros. — The metanephros, which is found as a separate kidney only in adult amniotes, probably is equivalent to the posterior portion of the mesonephros of the anamniotes, which it resembles greatly in its organogeny.

The region in which the metanephros arises is, like that in which the earlier kidneys are found, the mesomere or intermediate mesoderm. But in the posterior region of the body this mass is never segmented into separate nephrotomes. The first indication of metanephros formation is the appearance of an evagination from the dorsal surface of the mesonephric duct near the point at which the latter enters the cloaca. This evagination grows dorsally and then turns forward to become the **metanephric duct,** or **ureter,** in much the same manner as the collecting ducts of the mesonephros arose. The metanephric duct then sends out into the nephrogenous tissue evaginations which increase in length and branch repeatedly to form the **collecting tubules** of the metanephros. Around the distal end of each tubule, a small mass of the nephrogenous tissue condenses and acquires a lumen like the nephrocœles of the pronephroi and mesonephroi (Fig. 90; *1*, *2*). From these vesicles the **secretory nephric tubules** arise by a process of elongation and later fuse with the collecting tubules just described (Fig. 90; *3*, *4*). In each of the tubules a **capsule** develops for the reception of a glomerulus which later acquires a connection with a branch of the renal artery (Fig. 90; *4*, *5*). Development proceeds from the posterior

end toward the anterior, instead of in the opposite direction as in the earlier types of kidneys. The portion of the Wolffian duct nearest to the cloaca is absorbed by the latter so that the ureter has an opening into the cloaca separate from that of the mesonephric duct. From the region of the cloaca into which the ureters open is formed the **urinary bladder.** This is essentially

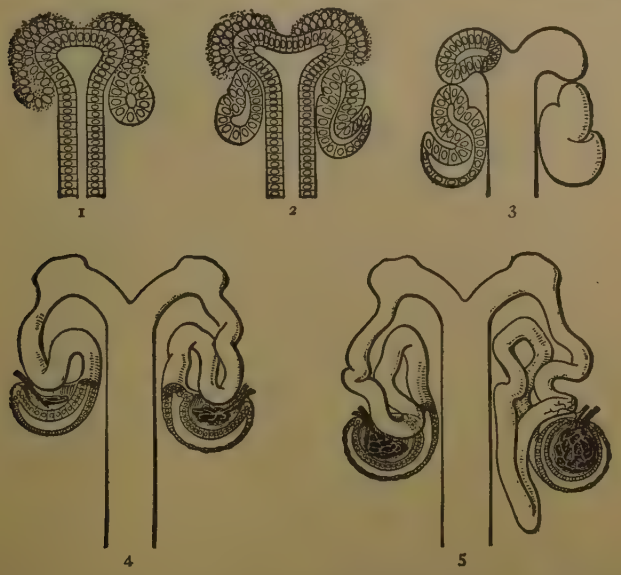

FIG. 90. — Diagrams to show development of metanephric tubules. The right half of each figure is the later one. (After Huber. From Arey, "Developmental Anatomy," copyright, 1924, by W. B. Saunders Co.)

an evagination of the cloaca, which in the amniotes continues to form the allantois as has been described in Chapter IV.

The later history of the nephroi and their ducts is considered in the next section, dealing with the gonads, with which the mesonephroi acquire a secondary connection.

The frog (*See also Chapter VIII*). — Three pronephric tubules are formed (somites II, III, IV) each with a nephrostome. The region of the cœlom into which these open is cut off ventrally by the development of the lungs and becomes the pronephric chamber. The glomeruli soon unite to form a **glomus.** Before

metamorphosis the pronephric tubes, and that portion of the duct into which they open, degenerate.

Mesonephric tubules appear in the nephrotomes (somites VII–XII). These have nephrostomes in early larval life; but at the time the pronephroi degenerate the portion of each mesonephric tubule next to the nephrostome (peritoneal canal) breaks away from the remainder of the tubule and fuses with the posterior cardinal vein. The mesonephros is the functional kidney of the adult, and the Wolffian duct, therefore, functions as the ureter.

The chick (*See also Chapter IX*). — About twelve pronephric tubules arise (somites V–XVI) beginning on the second day of incubation. Nephrostomes are formed, but glomeruli do not appear until the third and fourth days of incubation, at which time the pronephros is degenerating. The pronephric duct arises at the ninth somite.

Mesonephric tubules arise from the intermediate mesoderm between somites XII and XXX, the more anterior of which develop nephrostomes. The main part of the mesonephros, however, arises between somites XX and XXX, where the continued growth of the tubules causes the projection of this region into the cœlom as the Wolffian body. It is extremely doubtful whether the mesonephros ever functions as a kidney, as it begins to degenerate on the eleventh day.

The metanephros arises on the fourth day of incubation, from two primordia as usual, the intermediate mesoderm in somites XXXI–XXXIII, and an evagination of the mesonephric duct, comparable to the collecting ducts of the mesonephric tubules, which becomes the ureter, pelvis, and collecting ducts.

Man (*See also Chapter X*). — Pronephric tubules arise in somites VII–XIII, develop nephrostomes and glomeruli, but degenerate rapidly.

Mesonephric tubules appear in the intermediate mesoderm between the sixth cervical and fourth lumbar segments, but those of the cervical and thoracic segments soon degenerate. Nephrostomes are formed by the more anterior tubules but have only a transitory existence. The mesonephros does not function as a kidney.

The metanephros has a double origin as in the chick.

C. THE GENITAL ORGANS

The genital organs may be grouped into two classes: (1) the **primary genital organs,** or **gonads,** in which the germ cells develop; and (2) the **accessory genital organs,** or **gonoducts,** whose original function is the discharge of the germ cells from the body.

The gonads consist of the germ cells and the subordinate tissues, blood vessels, nerves, connective tissue, etc., which make up a large part of these glands. In an earlier chapter it has been shown that the primordial germ cells may first appear in the endoderm of the gut wall and thence migrate by way of the splanchnic mesoderm, dorsal mesentery, and peritoneum to their

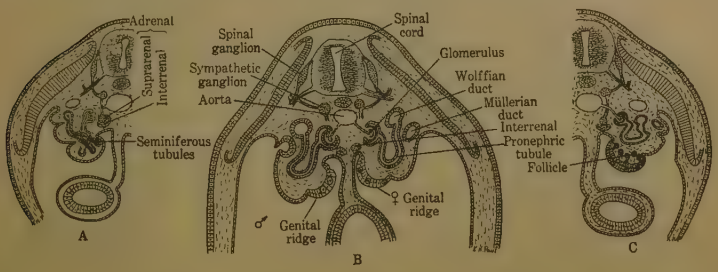

FIG. 91. — Diagrams to show the early development of the gonads. A, testis. B, genital ridge. C, ovary. (After Corning.)

definitive position in a thickening of the peritoneum on the mesial side of the nephrotomes. This thickening is called the **genital ridge** (Fig. 91*A*). A considerable body of evidence is accumulating to indicate that germ cells may also arise from the cells of the genital ridge itself.

The genital ridge is now invaded by mesenchymal cells, and projects into the cœlomic cavity. In some amphibia, a metameric arrangement corresponding to the myotomes has been recorded, but following this the segments unite. The anterior and posterior ends of the ridge degenerate, and the middle portion enlarges and is separated by a longitudinal groove from the mesonephros so that it hangs in the cœlom suspended by a fold of the peritoneum, known as the **mesorchium** in the male or the **mesovarium** in the female. The germ cells have by this time

become transformed into gonia (Chapter II) and the germ glands are known as gonads.

Within the gonads, the gonia come to lie in nests, close to the germinal epithelium. Tubular outgrowths from the nephric tubules of the mesonephros approach these nests. The later history of the gonads differs in the two sexes.

Testis. — In the male, the nests of spermatogonia become tubules which connect with the tubules growing in from the mesonephros (Fig. 91*B*). The testicular parts of these canals are known as the **seminiferous tubules,** the nephric portions as the **vasa efferentia.** The walls of the seminiferous tubules are composed of **spermatogonia** and **sustentacular cells** which act as nurse cells to the developing sperm. Between the tubules lie **septulæ** of mesenchymatous stroma containing so-called **interstitial cells** which probably arise from the germinal epithelium. It is because of the presence of these cells that the testis is sometimes spoken of as the " interstitial gland." Eventually, the tubules become separated from the surrounding germinal epithelium by the development of a mesenchymatous layer called the **tunica albuginea.**

Ovary. — In the female, the nests of oögonia become separate follicles (Fig. 91*C*) which never attain connection with the mesonephric tubules. These tubules consequently degenerate. A follicle consists of a single oögonium surrounded by follicle cells which may be compared to the sustentacular cells of the male. In the mammals, with the exception of the monotremes, the primary follicle enlarges through the multiplication of the follicle cells and secretes a fluid, the **liquor folliculi,** which collects to form a cavity, the **antrum folliculi,** separating the egg and a layer of investing cells, the **corona radiata,** from the outer wall of follicle cells, the **stratum granulosum,** except at a single point. In the **vesicular follicle** (**Graafian follicle**) (Fig. 92) the egg is supported, therefore, by a hillock of follicle cells known as the **cumulus oöphorus,** from which it is separated and expelled with the liquor folliculi at **ovulation,** by the rupture of the outer wall of the vesicular follicle. After ovulation, the follicle cells enlarge, multiply, and secrete a yellow substance (**lutein**), on account of which the resultant mass is called the **corpus luteum.** This body degenerates unless pregnancy occurs, in which case it produces

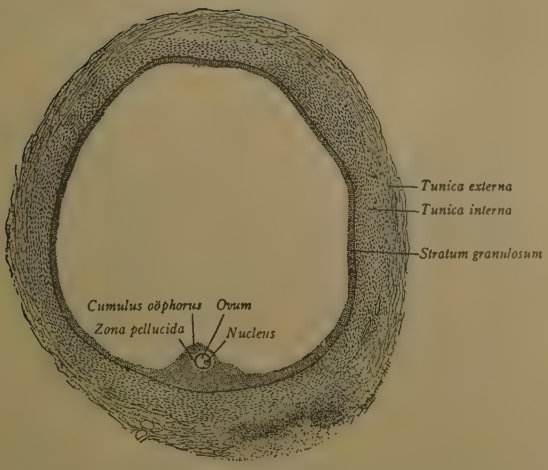

Fig. 92. — Human Graafian follicle. (After Prentiss. From Arey, "Developmental Anatomy," copyright, 1924, by W. B. Saunders Co.)

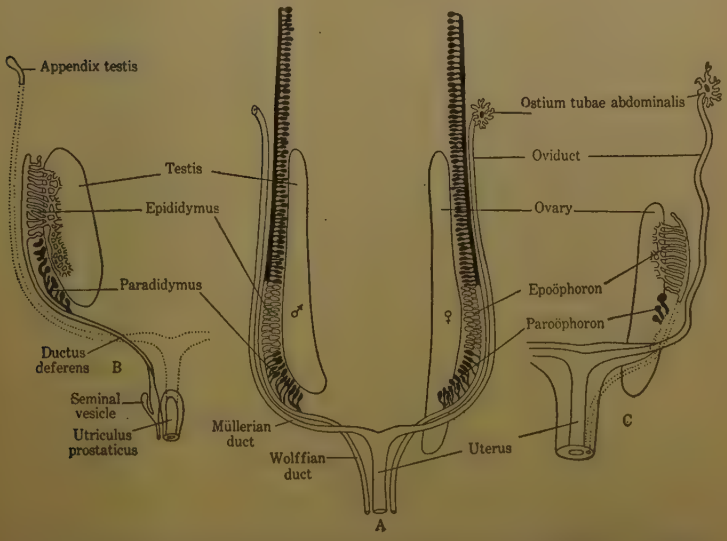

Fig. 93. — Diagrams to show the early development of the gonoducts. A, indifferent stage. B, male. C, female. (After Felix.)

a hormone necessary to the future development of the embryo. The tunica albuginea of the ovary develops much later than that of the testis but is also of mesenchymal origin.

The gonoducts. — The spermatozoa formed in the seminiferous tubules of the testis are discharged into the mesonephric tubules and thence make their way into the mesonephric duct, which accordingly becomes the male gonoduct. The ova, on the other hand, are discharged directly into the cavity of the cœlom whence they are received into a new tube, the **oviduct,** by means of an opening, the **ostium tubæ abdominale.** The mesonephric duct is often called the **Wolffian duct;** the oviduct is frequently called the **Müllerian duct.** Both ducts appear in every embryo (Fig. 93*A*), but the later histories of the two differ according to the sex.

The Wolffian duct. — In the male (Fig. 93*B*), the vasa efferentia toward the posterior end of the series become occluded, leaving only a few at the anterior end functional. These lose their renal corpuscles and shorten greatly. In the amniota, where the metanephros acts as the functional kidney, this anterior group becomes the **epididymis,** while the more posterior, non-functional vestige becomes the **paradidymis.** The mesonephric duct persists as the **ductus deferens.** At the point where the **ductus deferens** enters the cloaca, there develops a dilation, the seminal vesicle. In the female (Fig. 93*C*), the anterior portion of the mesonephros persists as the vestigial **epoöphoron,** and the posterior portion becomes the **paroöphoron.** Traces of the Wolffian duct sometimes persist, as in mammals, where this structure is known as **Gartner's canal.**

The Müllerian duct. — This canal arises in the elasmobranchs by the constriction of the pronephric duct into two tubes, of which the ventral becomes the Müllerian duct, while the dorsal tube becomes the Wolffian duct. The opening of the Müllerian duct into the cœlom, the ostium tubæ abdominale, is a persistent nephrostome. In all other vertebrates, this duct arises independently of and after the formation of the Wolffian duct, a fact possibly correlated with the delayed functioning of the oviduct as compared with the primary renal function of the Wolffian duct. In these vertebrates the duct arises in the mesomere lateral to the Wolffian duct and grows both forward and backward until the abdominal and cloacal openings are formed. The

latter, **ostium tubæ cloacale,** is not formed until late in embryogenesis. In the female (Fig. 93*C*), the posterior ends of the ducts are usually dilated as storage chambers, and not infrequently fuse to form a **uterus.** In the male (Fig. 93*B*), the Müllerian duct degenerates, but vestiges are to be found even in the adult, such as the **appendix testis** and **utriculus prostaticus** of man, which represent the anterior and posterior ends of the oviduct, respectively.

The œstral cycle. — In connection with the uterine mode of life characteristic of mammalian embryos, there are cyclical changes in the activity of the uterus, which as a whole are known as the œstral cycle. This is initiated by the **proœstrum,** marked by the congestion of the uterine blood vessels and uterine hæmorrhages (**menstruation**). The proœstrum is followed by the **œstrum,** a period in which the wall of the uterus offers the most favorable conditions for the implantation of the fertilized egg. It seems probable that this period corresponds to the time of ovulation in the ovaries, and is marked by sexual desire. If implantation does not take place, the **metœstrum,** during which the uterine wall is repaired, takes place. In mammals with a single breeding season, the metœstrum is succeeded by a *long* quiescent period, the **anœstrum.** In others, after a *short* quiescent period, the **diœstrum,** another proœstrum initiates a new œstral cycle immediately.

The external genitalia. — The genital organs so far considered, gonads and gonoducts, are common to all vertebrates and are sometimes spoken of as the **internal genitalia. External genitals** are found only in those animals in which fertilization is internal. These organs serve the function of transmitting or receiving the sperm at the time of fertilization. While internal fertilization is a phenomenon which has been observed in all classes of the vertebrates, it is obligatory in the amniotes.

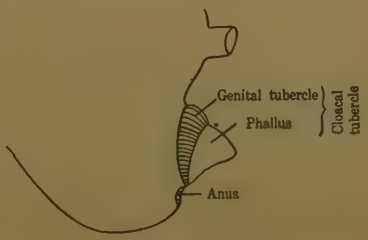

FIG. 94. — Diagram of the cloacal tubercle. (After Felix.)

Although the external genitalia differ in the sexes, they are

embryologically homologous. Two types are recognized, **duplex** and **simplex.** In the duplex type, characteristic of the sauropsids, sac-like extensions arise on each side of the cloaca, which in the male become the **hemipenes** or intromittent organ, while in the female they remain vestigial. In the simplex type, characteristic of mammals, a single median ectodermal prominence arises anterior to the cloacal aperture, to become the **phallus** (Fig. 94). In the male, the phallus enlarges and encloses the greater part of the urogenital sinus. In this way it becomes the **penis,** while the enclosed sinus becomes the **penile urethra.** In the female mammal, the phallus becomes the vestigial **clitoris,** while the sides of the urogenital sinus remain open as the **labia minora** which guard the opening of the urogenital vestibule.

TABLE VI

Homologies of the genital system

Male	Indifferent	Female
Testis	Gonad	Ovary
Spermatozoa	Gonocytes	Ova
	Gonoducts	
Epididymis	Mesonephros	Epoöphoron
Paradidymis		Paroöphoron
Ductus deferens	Mesonephric duct (Wolffian)	(Gartner's canal)
Appendix testis	Müllerian duct	Oviduct
		Uterus
Utriculus prostaticus		Vagina
Penis	Phallus	Clitoris

The frog (*See also Chapter VIII*). — The genital ridges arise soon after hatching. Sex can be distinguished at the time when the embryo is about 30 mm. in body length. The anterior portion of each genital ridge degenerates and becomes a **fat body.**

The Wolffian duct in the male acquires connection with the testis by means of some of the mesonephric tubules (vasa efferentia), and serves as the vas deferens as well as the ureter. A seminal vesicle is formed. A rudimentary Müllerian duct ap-

pears. In the female the Wolffian duct functions solely as a ureter while the Müllerian duct becomes the oviduct.

No external genitalia are developed.

The chick (*See also Chapter IX*). — The genital ridge arises with the Wolffian body as the urogenital ridge. Of this the anterior region gives rise to the gonad on the mesial side. Sex is not distinguishable until the seventh day of incubation. In the female, the right ovary develops only partially and finally disappears.

The Wolffian duct becomes the vas deferens, connected with the testis by vasa efferentia forming the epididymis. The persisting mesonephric tubules of the posterior region of the Wolffian body form a paradidymis. In the female a vestigial epoöphoron and paroöphoron represent these bodies respectively. The Müllerian ducts degenerate in the male without ever acquiring a cloacal exit. In the female the right Müllerian duct disappears while the left becomes the oviduct. The shell gland appears on the twelfth day of incubation, but the cloacal opening is not formed until the hen is six months old.

No external genitalia are formed, although hemipenes are formed in some other birds.

Man (*See also Chapter X*). — The genital ridge arises on the mesial side of the Wolffian body. Sex is not distinguishable until after the fifth week.

Each Wolffian duct functions as a vas deferens, and both epididymis and paradidymis are formed, as is a seminal vesicle at the distal end. In the female, epoöphoron and paroöphoron are formed, while some portion of the duct itself may persist as Gartner's canal. The Müllerian ducts become the uterine tubes, which unite at their posterior ends to form the uterus and vagina. The latter is partially closed by a semicircular fold, the **hymen,** where it enters the urogenital sinus. In the male, vestiges of the anterior end of each Müllerian duct persist as the appendix testis, while the posterior end is represented by the rudimentary utriculus prostaticus. The dilation of the bladder results in the inclusion of the ureters (metanephric ducts) in its walls. The gonoducts (Wolffian or Müllerian ducts) empty into the urogenital sinus posterior to the bladder, in a region which constricts to form the urethra. About this develop a

number of outgrowths which acquire cavities and form the **prostrate gland** in the male, and the **para-urethral glands** of the female.

The phallus becomes the penis of the male, the clitoris of the female. The penis elongates by the fusion of the lips of the urogenital opening, which in the female remain open as the labia minora. Folds arise on either side of the lips of the urogenital opening; in the male they fuse to form the **scrotum,** an external sac into which the testes descend; in the female they remain separate as the **labia majora.**

D. THE ADRENAL ORGANS

Closely associated with the nephric organs are the **interrenal glands,** which frequently become associated with the **suprarenal glands,** of ectodermal origin, to form the so-called **adrenals.** All are endocrine (or ductless) glands. The adrenals secrete a substance, **adrenalin,** which affects the heart, etc.

The interrenals. — These arise as paired thickenings of the splanchnic mesoderm mesial to the nephrocœles. In some of

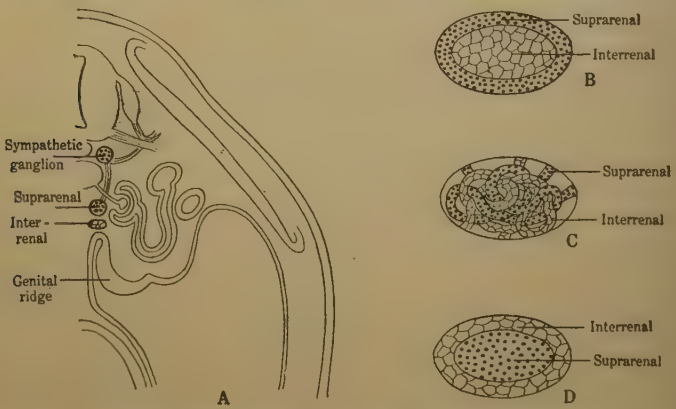

FIG. 95. — Diagrams of the adrenals. A, origin. B, amphibian complex. C, in chick. D, in mammals. (After Corning.)

the amphibia there are traces of a segmentation which is soon lost by fusion. There is no direct connection between the interrenals and the nephroi. These glands may fuse to form an elongate median organ or become associated with the suprarenals.

The suprarenals. — Although these glands are found in the vicinity of the nephroi, they originate from the sympathetic ganglia (ectodermal) as described in the following chapter. They are separate structures in the pisces, but unite with the interrenals in the tetrapods.

The adrenals (Fig. 95). — These glands are found only in the tetrapoda. In the amphibia the suprarenal portion of the gland is external to the interrenal portion. In the amniota, however, the interrenal substance surrounds the suprarenal.

E. THE VASCULAR SYSTEM

The vascular system is mesenchymatous in origin. It consists of separate cells, the **blood corpuscles,** floating in a fluid matrix, the **blood plasma,** in a closed system of interconnected tubes, the **blood vessels.** Some vessels become lined externally with muscle fibers, and in one locality this muscular development gives rise to a pulsating **heart** by means of which the blood is kept in circulation.

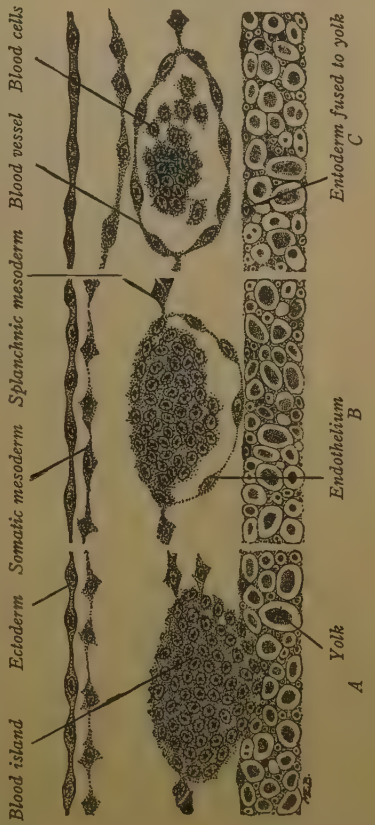

FIG. 96. — Transverse sections of the area vasculosa of a seven somite chick showing three stages in the development of blood islands. (From Arey, "Developmental Anatomy," copyright, 1924, by W. B. Saunders Co.)

Origin of the blood-vascular system. — Whether the mesenchyme of the blood-vascular system arises from the endoderm or from the mesoderm has always been a subject of controversy. The question seems a formal one, for in some vertebrates

the mesoderm takes its origin directly from endoderm while in others the two layers become differentiated from the common blastoderm simultaneously. The vascular mesenchyme is considered mesoderm whether it is separated from the endoderm early or late in histogeny. In any case, the first indications of the vascular system are found in the splanchnopleure as **blood islands.** The sum total of these is the **angioblast.**

The angioblast. — Blood islands (Fig. 96) first appear in the region of the yolk sac. In the macrolecithal vertebrates this is always in the extra-embryonic splanchnopleure. These blood islands are first found as local aggregates of mesenchyme. Later, the inner cells separate as **corpuscles,** while the outer ones form the endothelial lining of a **vesicle.** These vesicles anastomose with each other to form the **extra-embryonic vitelline circulation.**

The blood corpuscles. — The first corpuscles formed are the inner cells of the blood islands. Later corpuscles are budded off

Fig. 97. — Blood cells from human embryos. A, hæmoblasts. B, ichthyoid blood cells. C, D, E, sauroid blood cells. F, erythrocytes. (After Prentiss. From Arey, "Developmental Anatomy," copyright, 1924, by W. B. Saunders Co.)

from the walls of the capillaries into their cavities. Mesenchymal cells in regions where the capillary network is forming may develop into blood corpuscles and enter the blood stream. These first corpuscles are the **hæmoblasts.**

Hæmoblasts become differentiated into the different types of blood corpuscles in the following blood-forming centers: (1) the yolk sac; (2) the embryonic capillaries; (3) the liver, the

spleen, and the lymph glands; (4) the bone marrow. In the adult the lymph glands give rise to **lymphocytes,** and the bone marrow to all types of corpuscles.

The **erythrocytes,** or red corpuscles, are distinguished by the presence of **hæmoglobin** which gives them their color. In the anamniotes the erythrocytes have a large vesicular nucleus with granular chromatin and a distinct cell membrane. In the sauropsida, the erythrocytes have a small compact nucleus. The mammalian erythrocyte is distinguished by the absence of the nucleus in the adult. In the development of mammals there is a succession of erythrocytes: first the anamniote type; then the sauropsid type; and finally the mammalian erythrocyte, which is produced by the extrusion of the nuclei from the blood cells of the sauropsid type (Fig. 97).

The **leucocytes,** or white corpuscles are of many types, for a discussion of which the reader is referred to the text-books on histology. The preponderance of evidence indicates that these, like the erythrocytes, are derived from the hæmoblasts.

Origin of the intra-embryonic vessels. — The first embryonic blood vessels (Fig. 98) are the **vitelline veins** which appear at the ventro-lateral margins of the fore-gut. The anterior extensions of these vessels fuse in front of the liver to form the **heart,** then separate as the **ventral aortæ,** which bend up around the pharynx in the hyomandibular arch as the **first aortic arches,** and continue backward as the **dorsal aortæ.** These fuse at a very early stage as the **dorsal aorta,** from which branches are sent to each myotome and to the vitelline circulation. The posterior ends of the vitelline veins fuse in small-yolked forms, such as the frog, to form a **subintestinal vein** which continues back to the tail. In large-yolked forms like the chick, the vitelline veins are widely separated and brought into connection only by the **sinus terminalis** which makes a circuit of the area vasculosa. The vitelline veins are the ventral venous channels of the **splanchnic circulation.** A dorsal set of vessels soon originates independently to form the **somatic venous circulation.** The first of these to appear are the **anterior cardinal veins** of the head. A similar pair, the **posterior cardinal veins,** arise in connection with the nephric region. These, however, do not discharge their contents directly into the heart but into the **anterior cardinals.** The portions of the origi-

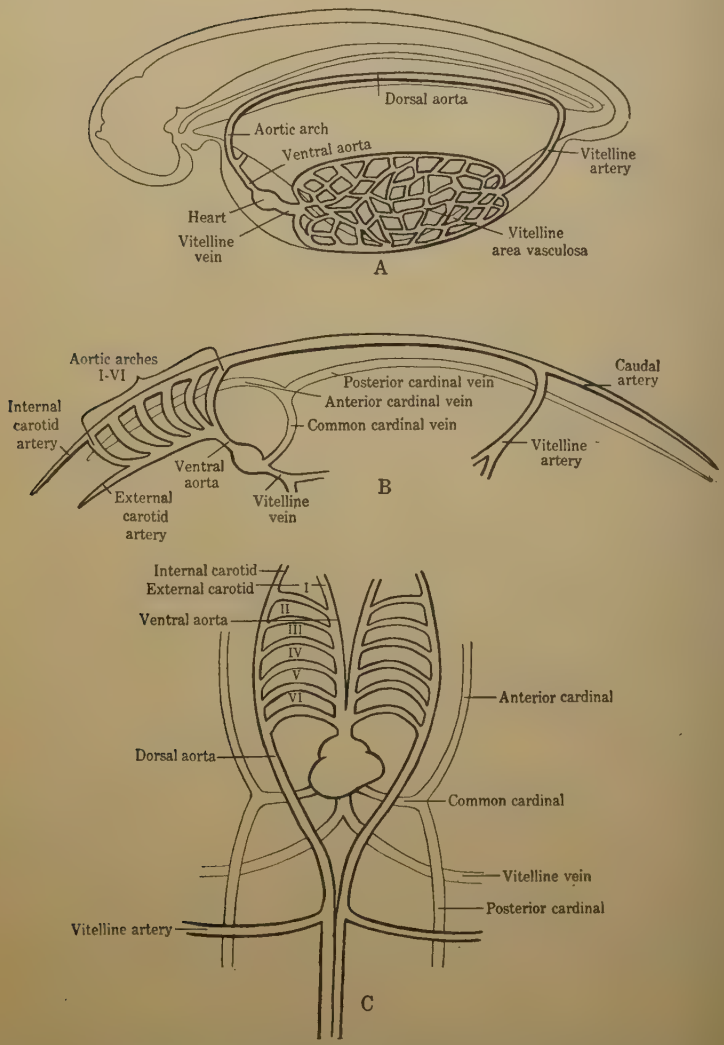

FIG. 98. — Diagrams of primitive embryonic circulation. A, early stage. B, main vessels, side view. C, same in plan.

nal anterior cardinals proximal to this juncture with the posterior cardinals are now called the **common cardinal veins.**

The heart. — Although the heart is primitively a paired organ, we have seen that the two primordia are soon fused into a single median tube connected with the ventral aortæ in front, and the vitelline veins (and later the common cardinals) behind. Around the **endocardial lining** there develops a coat of muscle fiber which later becomes striated to form the **myocardium.** Outside this is a lining of splanchnic mesoderm which forms the **epicardium,** continuous with the lining of a part of the cœlom surrounding the heart, which will later be cut off by the septum transversum to form the pericardium. In this the heart is suspended by a dorsal and a ventral mesentery known respectively as the dorsal and ventral mesocardia.

The later history of the heart is one of growth and subdivision into special chambers. Because the local growth of the heart

FIG. 99. — Diagrams to show five stages in the development of the heart. See text for explanation.

is limited by the anterior and posterior walls of the pericardium and by the mesocardia in which it is suspended, any extension in length must be accompanied by coiling. The primary flexure of the heart is toward the right, thus changing the shape of the organ from a straight tube to a **C**-shaped one. Further growth results in the twisting of the heart into the shape of an **S.** Still later, the original posterior loop of the S is pushed forward and dorsad so that it comes to lie above the morphologically anterior end (Fig. 99).

The original chambers of the heart are produced by local dilations, of which the most posterior is the **sinus venosus;** next to

this is the **atrium**; in front of this, the **ventricle**; and finally, the **bulbus arteriosus.** The sinus is the chamber into which the primitive veins enter; the atrium is a thin-walled distensile chamber; the ventricle is a thick-walled, muscular, pulsating pump; while the bulbus is the chamber from which the blood enters the primitive arteries.

These chambers undergo different changes in the various types of vertebrates. Of these, the most important is a progressive differentiation, completed in the mammals and birds, of the atrium and ventricle into separate right and left halves, of which the right side receives venous blood from all parts of the body and transmits it to the lungs for respiratory exchange. From the lungs the blood is returned to the left side of the heart and thence conveyed to all parts of the body.

The arteries (Fig. 100). — The ventral aortæ fuse into a single median tube sending branches into each of the visceral arches.

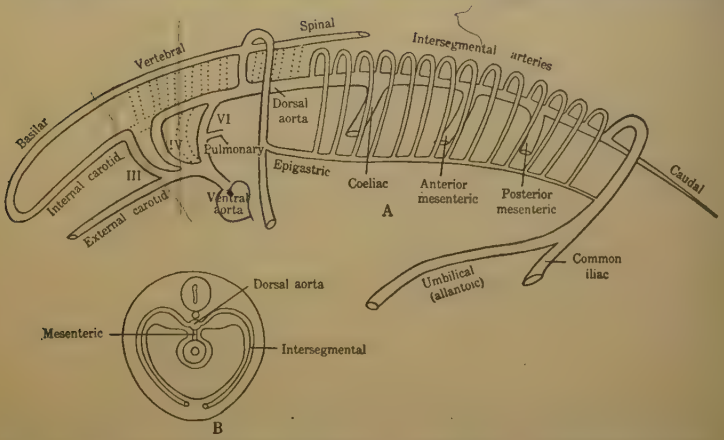

FIG. 100. — Diagrams of the major arteries. A, side view. B, cross section through intersegmental and mesenteric arteries.

These branches, which unite with the dorsal aortæ, are usually six in number and are known as the **aortic arches.** Anterior to these the ventral aortæ continue forward as the **external carotid arteries.** Similar forward extensions of the dorsal aortæ are known as the **internal carotid arteries.** In the region of the aortic arches the dorsal arteries remain separate as the **aortic**

roots. Behind them, as has been mentioned, the paired vessels fuse as the median dorsal aorta.

The aortic arches. — In larvæ breathing by means of external gills, a loop from each aortic arch grows out into the gill developing on the visceral arch with which it is associated. These loops are short-circuited when the external gills disappear.

In forms with internal gills, each aortic arch breaks up into capillaries in the demibranch and becomes divided into a ventral **afferent branchial artery** and a dorsal **efferent branchial artery.**

In vertebrates with a pulmonary respiration, aortic arches I and II, in the mandibular and hyoid arches, respectively, disappear. Arch III, in the first branchial arch, persists as the

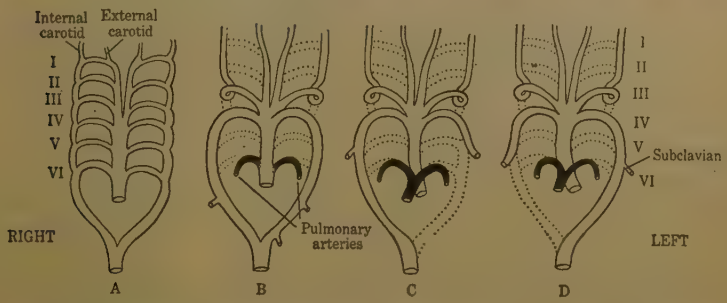

FIG. 101. — Diagrams of the aortic arches. A, primitive. B, in frog. C, in chick. D, in man. (After Kingsley, "Comparative Anatomy of Vertebrates," copyright, 1926, by P. Blakiston's Son and Co., modified.)

connection between the internal and external carotid arteries, while the dorsal aorta between arches III and IV disappears. Arch IV becomes the **systemic arch** connecting the dorsal and ventral aortæ (Fig. 101*B*). In birds (Fig. 101*C*), the arch on the left side disappears; in mammals (Fig. 101*D*), that on the right degenerates. Arch V is greatly reduced and frequently disappears or has at most a vestigial and transitory existence. From arch VI there grow back to the lungs the **pulmonary arteries.** The portion of the sixth arch distal to the pulmonary arteries is reduced in caliber and is known as the **ductus arteriosus.** It becomes occluded and degenerates in all the amniotes except some few reptiles.

Intersegmental arteries. — From the dorsal aortæ are given off small branches between the myotomes (Fig. 100*B*). Some of these **intersegmental arteries** persist as the **intervertebral arteries.** The more anterior ones become united on either side by a dorsal longitudinal **vertebral artery.** These vertebrals subsequently fuse to form an anterior **basilar artery** which divides behind the pituitary, the two halves uniting with the internal carotid on either side. The posterior halves of the vertebral arteries fuse to form the **spinal artery** which runs back beneath the spinal cord. In the region where the anterior limb buds are developing, intersegmental arteries grow out, to give rise to the **subclavian arteries.** Similarly, in the region of the pelvic limb buds, intersegmental arteries give rise to the **iliac arteries.** In the amniota, the **allantoic arteries** grow out from the iliac arteries into the walls of the allantois. These become so important that for some time it appears as though the iliac arteries were derived from the allantoics instead of the reverse. These allantoic arteries, which degenerate at the time of birth, are known as the **umbilical arteries** in mammals as they traverse the umbilical cord and supply the placenta.

Other important intersegmental arteries become the **renal arteries** of the nephroi and the **genital arteries** of the gonads.

Mesenteric arteries. — From the dorsal aorta, a number of ventral branches, originally paired, but soon fused to become median vessels, pass down the dorsal mesentery. They unite with the capillaries of the yolk sac which they supply with blood. Later, some of them develop branches over the alimentary canal which persist after the loss of the yolk sac as the **cœliac** and **mesenteric arteries.**

The veins. — There are two primitive venous systems to be considered: the **somatic,** or cardinal, system; and the **splanchnic,** or vitelline, system. The cardinal system is replaced by a caval system; the vitelline system becomes transformed into a portal system. Finally, the pulmonary veins are to be considered. In general, the history of these transformations may be summed up in the statement that the primitive independent venous systems become transformed into a system wherein there is developed an accompanying vein for every artery.

The vitelline veins (Fig. 102). — These vessels, and their continuation, the subintestinal vein (in small-yolked forms), are the first vessels formed in the embryo. In the amniota, two veins grow out from these into the wall of the allantois to become the allantoic veins of the sauropsida (umbilicals of mammals). In man, the umbilical veins actually appear before the vitelline veins, and conditions are apparently reversed.

It has been noted previously that the vitelline veins pass around the liver on their way to the heart. As the liver enlarges,

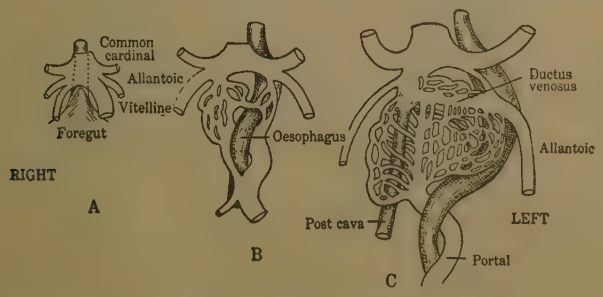

FIG. 102. — Diagrams to show three stages in the development of the hepato-portal veins. (After Hochstetter.)

it surrounds the vitelline veins, and these become broken up in the liver tissue to form a great capillary network. In the amniota, the allantoic (umbilical) veins are similarly absorbed. The proximal portions of the vitelline veins, from the liver to the sinus venosus, are now known as the **hepatic veins**; the distal portions are called the **portal veins.** Of these, the right degenerates; the left for a time maintains a direct connection through the liver to the hepatic veins, known as the **ductus venosus.** This connection disappears at birth in the mammalia. After the disappearance of the yolk, the portal vein and its tributaries, of which the most important is the **mesenteric vein,** carry blood from the alimentary canal to the liver.

The anterior cardinal veins. — The original plan of the cardinal system is that of an H in which the upper limbs represent the anterior cardinals; the cross-bar the common cardinals, with the heart in the middle of the cross-bar; and the lower limbs represent the posterior cardinals (Fig. 103). The anterior cardi-

nals arise as a drainage system for the blood passing into the head from the carotid arteries.

The anterior cardinals are often called the **superior jugular veins.** From these, parallel veins, known as the **inferior jugular veins,** branch off in the ventral region of the head. Veins from the vertebral region (**vertebral veins**) and from the pectoral appendages (**subclavian veins**) soon develop. In most vertebrates the common cardinals and the proximal portion of the anterior cardinals, i.e., up to the point where these tributary veins diverge, persist as the **anterior caval veins.** In some mammals, a

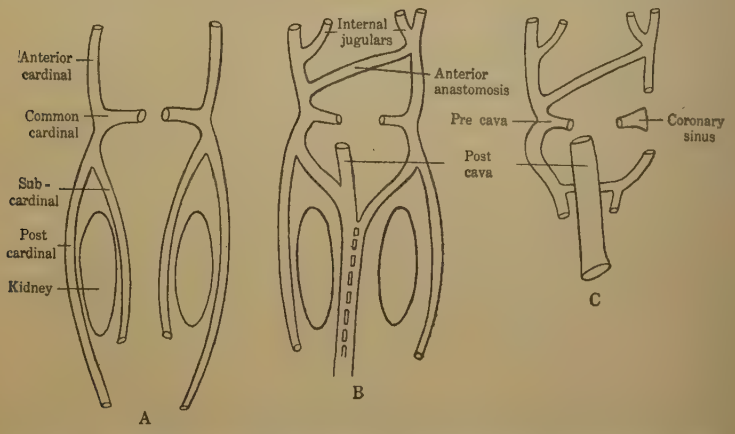

FIG. 103. — Diagrams showing three stages in the development of the somatic veins.

cross-connection occurs between the anterior cardinals, after which the portion of the left anterior cardinal proximal to the anastomosis, and the left common cardinal, degenerate, leaving only the **coronary vein** from the wall of the heart, while the corresponding vessels on the right side persist as the **precaval vein.**

The posterior cardinals. — The posterior cardinals lie dorsal to the mesonephroi which they drain. Beneath the mesonephroi are developed a set of parallel veins called the **subcardinal veins.** In the lower vertebrates they arise as tributaries of the posterior cardinals and extend backward into the tail as the **caudal vein.** Later, they lose direct connection with the parent

vessels and return blood from the tail region to the mesonephroi as the **advehent renal veins.** The posterior portions of the subcardinals fuse as the **interrenal vein,** which acquires a secondary connection with the hepatic vein, and persists as the **postcaval vein.** In the amniota the postcaval arises partly from a new vein growing forward to the hepatic veins, partly from appropriated portions of the post cardinals and subcardinals, and eventually replaces the posterior cardinals, so that the only blood vessels entering the right side of the heart are (1) the precaval returning blood from the head, pectoral region, and appendages, and (2) the postcaval returning blood from the trunk and pelvic appendages as well as all blood from the alimentary canal conveyed *via* the hepato-portal system.

The pulmonary veins. — These enter the left atrium and are new vessels which grow backward from the heart to the developing lungs.

The lymphatic system. — This system serves to return to the veins the blood plasma which has escaped from the capillaries (Fig. 104). It contains white blood corpuscles of the amœboid type (lymphocytes) which have the power of making their way through the capillary walls. The lymphatics apparently originate as inter-cellular spaces in mesenchyme which later become confluent and acquire a limiting endothelium. Like the blood vessels, the lymphatic capillaries anastomose and form larger vessels which drain into the veins. The walls of these central vessels are often muscular, and localized areas known as **lymph hearts** are found. So, too, localized distensible sacs, the **lymph sacs,** are not unknown. Some of these become **lymph glands.** The spleen, already alluded to in the section on mesenteries (page 135), is a **hæmolymph gland** in which both lymphocytes and erythrocytes are proliferated.

The frog (*See also Chapter VIII*). — In the frog (Fig. 105), the primordia of the vitelline veins first appear and grow together as a loose aggregate of cells in front of the liver. Around this the splanchnocœle grows in from right and left to form the pericardium. Meantime the primordium of the heart endocardium develops from the loose aggregate of cells referred to above. The inner wall of the splanchnocœle becomes the myocardium, while the outer wall becomes the pericardium. The

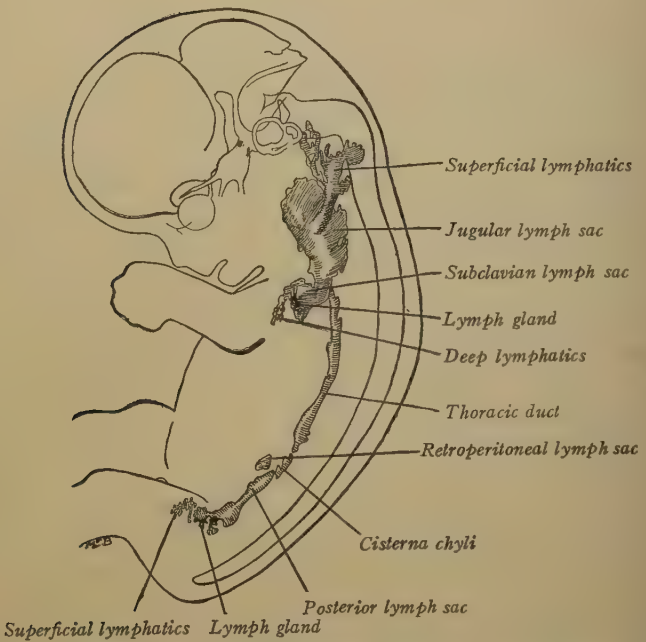

FIG. 104. — Diagram of the primitive lymphatic vessels in a human embryo of two months. (After Sabin. From Arey, "Developmental Anatomy," copyright, 1924, by W. B. Saunders Co.)

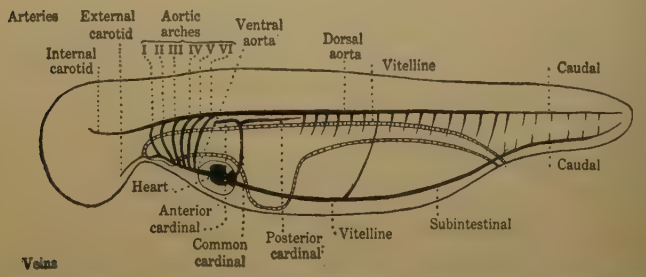

FIG. 105. — Diagram of the embryonic circulation of the tadpole. (After Kingsley, "Comparative Anatomy of Vertebrates," copyright, 1926, by P. Blakiston's Son and Co., modified.)

atrium is divided by an interatrial septum into two auricles, right and left. The ventricle remains a single chamber.

Aortic loops develop in the external gills, to form arches III, IV, and V. After the disappearance of the external gills, the ventral limb of the loop becomes the afferent branchial artery, while the dorsal limb becomes the efferent branchial artery. A similar differentiation takes place in arch VI. With the loss of branchial respiration, arch III becomes the proximal portion of the carotid arteries, arch IV the systemic arch which persists on both sides, and arch V disappears, while from arch VI arise vessels which carry blood to both the lungs (pulmonary arteries, Fig. 101*B*) and skin (cutaneous arteries).

The vitelline veins anterior to the liver fuse to become the hepatic vein; posterior to the liver, the right vitelline vein disappears, the left becomes the portal vein. The subintestinal vein represents the sinus terminalis of the chick. The anterior cardinal veins become the internal jugular veins; the common cardinals, the anterior venæ cavæ. The posterior cardinal veins fuse between the mesonephroi, and a new vein grows back from the hepatic vein to the right postcardinal, to form the postcaval vein. The posterior cardinals, anterior to their junction with the postcaval, degenerate. The supracardinal veins form the renoportal veins carrying blood from the iliac veins to the kidneys.

The chick (*See also Chapter XI*). — In the chick (Fig. 106), the endocardium of the heart arises as the forward extension of the vitelline veins, which soon fuse as the pericardial primordia are brought together beneath the head. The myocardium is formed as in the frog. The right and left halves of the heart are completely separated by three septa: the **septum aorticopulmonale,** which divides the bulbus into a chamber on the right leading to the pulmonary arteries and one to the left leading to the dorsal aorta; the **interventricular septum,** which divides the ventricle; and the **interatrial septum,** which divides the atrium into two auricles. This separation is completed at the end of the first week of incubation. The sinus venosus is incorporated in the right auricle.

Six aortic arches are formed: I and II disappear on the third and fourth days of incubation; III forms the proximal portion of the internal carotid artery; IV disappears on the left side

but persists as the systemic arch on the right; V disappears; the pulmonary arteries arise from VI, but the distal portion of the right arch remains as the ductus arteriosus until the chick hatches (Fig. 101*C*).

The vitelline veins unite behind the sinus venosus to form the **meatus venosus** which becomes the hepatic vein. The mesenteric vein becomes the portal vein, and the vitelline veins dis-

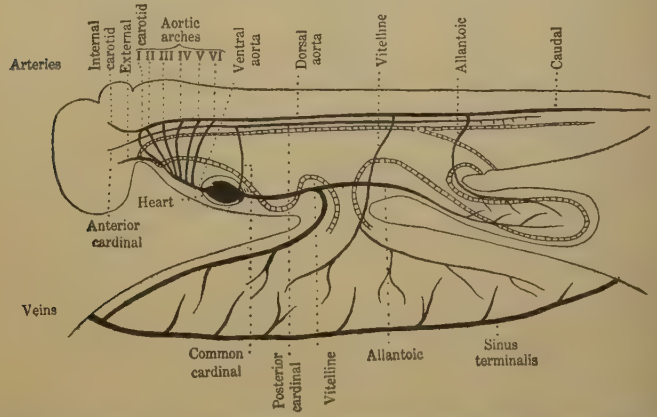

FIG. 106. — Diagram of the embryonic circulation of the chick. (After Kingsley, "Comparative Anatomy of Vertebrates," copyright, 1926, by P. Blakiston's Son and Co., modified.)

appear at hatching. The allantoic veins grow backward from the common cardinals to join the capillaries of the allantois; the right allantoic degenerates on the fourth day, and the left acquires a new connection with the meatus venosus, by way of the left hepatic vein. The allantoic vein degenerates at hatching. Two anterior caval veins are formed from the proximal portions of the anterior cardinals and common cardinals. The posterior vena cava arises from a branch of the meatus venosus which grows back to meet the right subcardinal vein, the fused subcardinals which carry blood from the mesonephroi, and the renal veins which develop in connection with the metanephroi. The anterior ends of the postcardinals disappear, while the posterior ends form the renoportals of the mesonephros and, after its degeneration, the common iliac veins, which pass directly to the postcaval.

Man (See also Chapter X). — The heart arises in man (Fig. 107) much as in the chick; but the subsequent partitioning of this organ into right and left halves is more complicated, for two atrial septa are formed. The ventricle is separated by an interventricular septum, and the bulbus is divided by two septa which unite to form the septum aortico-pulmonale. The sinus venosus is incorporated in the right atrium.

The aortic arches are formed and have the same history as those of the chick, with the exception that it is the left fourth aortic arch which becomes the systemic arch (Fig. 101*D*).

The anterior portion of the right vitelline vein becomes the hepatic vein; the portal arises from the posterior portion of the

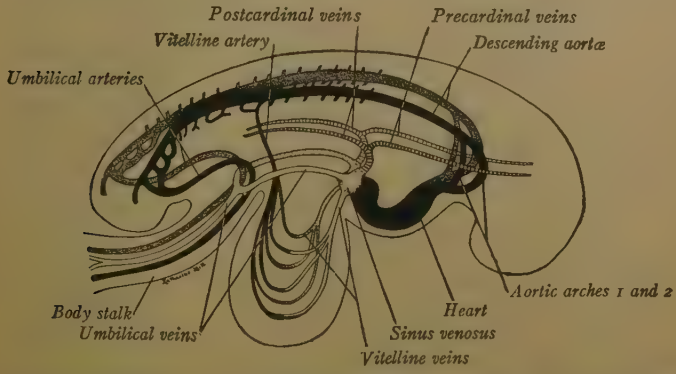

FIG. 107. — Diagram of the embryonic circulation of man. (After Felix. From Arey, "Developmental Anatomy," copyright, 1924, by W. B. Saunders Co.)

vitelline veins anterior to their junction with the mesenteric vein. The anterior cardinals are united by an anastomosis (left innominate vein), and the left common cardinal disappears with the exception of the coronary sinus. The right common cardinal, together with that portion of the anterior cardinal as far as the branching of the left innominate, becomes the anterior vena cava. The posterior vena cava is a complex formed from (1) a branch of the hepatic vein, (2) the anterior portion of the fused subcardinals, (3) part of the fused supracardinals, and (4) the fused posterior portion of the postcardinals. The anterior portions of the posterior cardinals separate from these veins, unite by means

of an anastomosis, and drain into the right common cardinal or precaval to form the azygos vein. Of the umbilical veins, the left only persists with a direct connection through the liver by means of the ductus venosus. At birth this duct closes and the umbilical vein disappears.

F. THE SKELETON

The skeleton of vertebrates consists of a system of supporting and protecting elements developed from mesenchyme. These elements pass through several conditions in later development. The primordia of the skeletal elements are preformed in connective tissue. These become transformed into cartilage, a process known as **chondrification,** through the activities of specialized cells, the **chondrioblasts.** Cartilage in turn is trans-

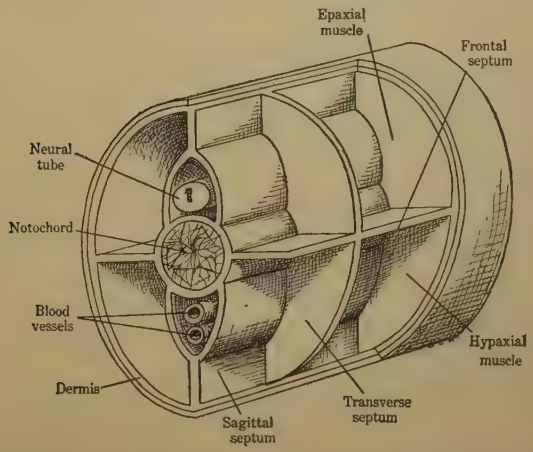

FIG. 108. — Diagram of the skeletogenous regions in the vertebrate tail. (After Kingsley, "Comparative Anatomy of the Vertebrates," copyright, 1926, by P. Blakiston's Son and Co., modified.)

formed into bone, through the action of **osteoblasts,** the process being known as **ossification.** Bones that pass through these three stages are known as cartilage bones. In the formation of some bones, the cartilaginous stage is omitted; these are known as membrane bones. Both cartilage and bone are typically surrounded by a membrane of mesenchyme which is called the

perichondrium or **periosteum,** as the case may be. The separate elements of the skeleton are connected with each other by ligaments, by cartilage, or in a bony union.

Skeletogenous regions. — The principal regions where skeleton may be formed in the vertebrate body (Fig. 108) are (1) the dermis of the skin, (2) the median sagittal planes between the myotomes on the dorsal and ventral sides of the body, (3) the right and left frontal planes between the dorsal and ventral muscle masses, (4) the transverse planes between the myotomes, (5) around the notochord, neural tube, and axial blood vessels, (6) in the visceral arches, and (7) in the paired appendages. Skeletal elements formed in (1) are called the **dermal skeleton;** those formed in (2) to (5), the **axial skeleton;** those formed in (6), the **visceral skeleton;** and those formed in (7), the **appendicular skeleton.** The skull contains elements from all but the appendicular skeleton.

The dermal skeleton. — The dermal skeleton in its most primitive form was undoubtedly the exoskeleton of the extinct placo-

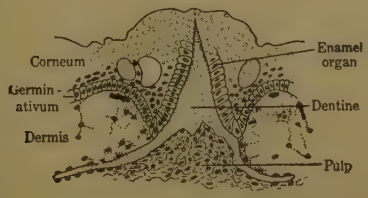

FIG. 109. — Section of developing scale of Acanthias. (After Kingsley, "Comparative Anatomy of the Vertebrates," copyright, 1926, by P. Blakiston's Son and Co., modified.)

derms. Among living vertebrates the most primitive example of derm bones are the placoid scales (Fig. 109) of the chondrichthyes which are formed in exactly the same way as teeth (Chapter V). In the dermal skeleton two types of bones are distinguished. The **investing bones** (**dermal plates**) probably arose from the coalescence of the bony trabeculæ at the bases of the placoid scales, and serve to envelop regions of the head and trunk. The **substituting bones** become so closely allied with the cartilaginous bones as to become fused with them or even to replace them in ontogeny. Many of the cranial bones are of this type. They

may be distinguished by the fact that they do not pass through a cartilaginous stage in development.

The axial skeleton. — The primitive axial skeleton is the notochord, which is laid down by precociously segregated mesoderm.

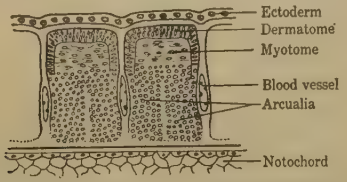

FIG. 110. — Arcualia separating in myotomes of Sceloporus. (After Kingsley, "Comparative Anatomy of the Vertebrates," copyright, 1926, by P. Blakiston's Son and Co., modified.)

Around this a connective tissue sheath is formed by mesenchymal cells. The mesenchyme from each sclerotome now forms four little blocks, the **arcualia** (Fig. 110), two dorsal to the notochord and two ventral, from which the **arches** and **centra** of the vertebræ are formed, as well as the primordia of the **ribs.** The posterior arcualia of each somite unite with the anterior arcualia of the succeeding myotome to form the definitive vertebra, which thus comes to lie at the point of separation between two myotomes. Eight elements are thus concerned with a single vertebra: right and left dorsal arcualia from the anterior half sclerotome, and from the posterior half sclerotome, and the corresponding ventral elements.

FIG. 111. — Ossification centers in human vertebra and ribs. (After Kollmann.)

The vertebræ. — In the prevertebral masses so formed appear centers of chondrification, one on each side of the spinal cord and one or more below the cord. These form, respectively, the neural arch and the centrum of the vertebræ (Fig. 111). In the

tail region, two centers of chondrification arise below the centrum, enclosing the caudal prolongation of the dorsal aorta, and form a hæmal arch. With the condrification of the vertebræ the notochord disappears in all but the most primitive vertebrates, persisting only between the vertebræ as **nuclei pulposi** of the intervertebral discs. Finally, in the osteichthyes and tetrapods, the vertebræ become ossified, and the spines, zygapophyses, and other differentiations are developed.

The ribs. — Except in the caudal region, lateral processes arise from the vertebral primordia and grow out into the myosepta. They later become cartilaginous, and finally true bone. These are the ribs, of which there are two types, dorsal and ventral, distinguished according to the part of the vertebra from which they originate.

The sternum. — The sternum, or breast bone, arises in the amphibia from the coalescence of two longitudinal bars of cartilage, which later articulate with the coracoids of the pectoral girdle, but do not come in contact with the ribs. In the amniote, the sternum arises from the fusion of the ventral ends of the anterior rib rudiments. In this way there arise two longitudinal bars, from which the unpaired sternum is formed by fusion along the mesial line (Fig. 112).

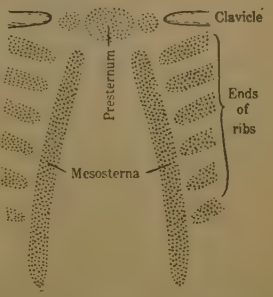

FIG. 112. — Scheme of mammalian sternum. (After Kingsley, "Comparative Anatomy of Vertebrates," copyright, 1926, by P. Blakiston's Son and Co., modified.)

The skull. — The **skull** is a complex of skeletal elements, arising from the **chondrocranium,** or primitive cranium of cartilage bones, which is derived in part from the protective covering of the brain, or **neurocranium,** and in part from the supporting elements of the visceral arches, or **splanchnocranium.** This is supplemented by numerous investing and substituting bones from the original dermal skeleton, or **dermocranium.**

Neurocranium. — The neurocranium arises from the head mesenchyme which, as has been said, can not be traced to any definite somites. In this mass, which completely invests the brain and sense organs, definite centers of chondrification appear,

from which develop areas comparable to the areas of the cartilage skull of the chondrichthyes (Fig. 113). If the notochord be used as a point of orientation, on either side of it is found a **parachordal bar.** In front of each of these is a separate rod; these are the **trabeculæ.** Between the two parachordals and around the notochord, the **basilar plate** arises to form the floor of the epichordal brain. The trabeculæ also fuse in front, to form the **ethmoid plate** which is the floor of the prochordal brain, but remain separate at their posterior ends to form an opening through which the pituitary projects downward. In front of the ethmoid plate the trabeculæ grow forward as the **cornua.** Dorsal to each

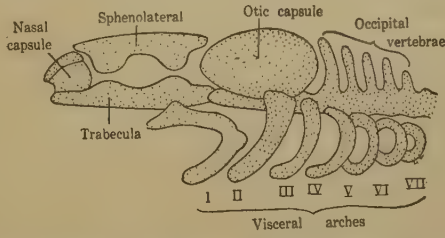

Fig. 113. — Chondrocranium of Acanthias. (After Kingsley, "Comparative Anatomy of Vertebrates," copyright, 1926, by P. Blakiston's Son and Co., modified.)

trabecula, another longitudinal bar, the **sphenolateral,** arises. Between these two bars the cranial nerves make their way to the exterior.

Around each of the major sense organs a cartilaginous capsule develops. The **olfactory capsules** unite with the cornua, ethmoid, and sphenolaterals. The **optic capsule** rarely develops fully, usually persisting in the connective tissue stage as the **sclera** of the eyeball. The **otic capsule,** however, becomes completely chondrified and unites with the parachordals and the latero-sphenoids. Between the two otic capsules and sphenolaterals arises a **dorsal plate** which forms a roof for the epichordal brain. Finally, one or more neck vertebræ are consolidated with the caudal end of the neurocranium to form the **occipital region.**

The splanchnocranium. — The alimentary canal in the head region consists of the mouth, oral cavity, and pharynx, the walls of the pharynx being penetrated by the visceral clefts. As there is no cœlom in this region, the lateral mesoderm is not divided but

gives rise to mesenchyme which foreshadows the cartilaginous bars to support the wall of this part of the body. These visceral arches are the mandibular, hyoid, and four (or more) branchial arches. The mandibular arch divides into dorsal and ventral portions, of which the dorsal portion becomes the **pterygoquadrate cartilage** (upper jaw of chondrichthyes) while the ventral portion becomes the **meckelian cartilage** (lower jaw of chondrichthyes). The hyoid arch divides into a dorsal **hyomandibular cartilage,** and a ventral **hyoid cartilage** which is usually divided into several centers of chondrification. The hyomandibular acts as a suspensory element for the jaws in the fish, and becomes in higher forms the **columella** or **stapes** of the ear. The hyoid gives rise to the support of the tongue. The branchial arches are usually divided into four parts and act as gill supports in the lower vertebrates and disappear or become **laryngeal cartilages** in the higher forms.

Ossification of the chondrocranium. — The limits of this text will not permit of an enumeration of all the bones formed from

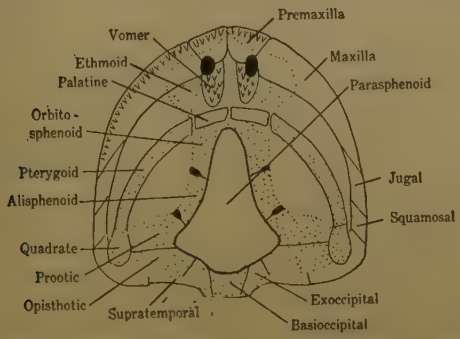

FIG. 114. — Cranial elements. Ventral view. Chondrocranium shaded. Dermal elements outlined. (After Kingsley, "Comparative Anatomy of Vertebrates," copyright, 1926, by P. Blakiston's Son and Co., modified.)

the chondrocranium (Figs. 114, 115, 116). They may be grouped, however, as follows: (1) the **occipitals,** formed from the occipital vertebræ; (2) the **sphenoids,** arising from the parachordals, basilar plate, trabeculæ and latero-sphenoids; (3) the **ethmoids,** from the ethmoid plate and nasal capsule; (4) the **otics,** from the otic capsule. The pterygoquadrate bar gives rise to the **ptery-**

goid bones and the **quadrate** (which in mammals becomes the **incus**). The meckelian cartilage gives rise to the **articular** bone at its distal extremity. This becomes the **malleus** of the mammals. The remainder of the meckelian persists as cartilage. In

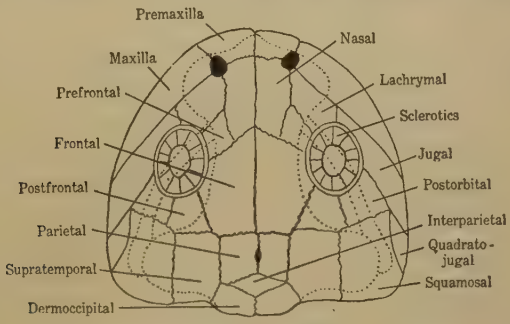

FIG. 115. — Cranial elements. Dorsal view. Compare Fig. 111.

the hyoids and the branchials, bones are formed which retain the names of their cartilaginous predecessors.

The dermocranium (Figs. 114, 115, 116). — The derm bones which invest and, to some extent, supplant the elements of the chondrocranium are too numerous to be more than mentioned here. The dorsal derm bones are, from front to rear, the **nasals, frontals,** and **parietals,** together with a number of smaller bones which appear in variable quantity in the different classes. The principal lateral elements, from front to rear, are the **premaxillæ, maxillæ, jugals, quadratojugals,** and **squamosals.** The floor of the chondrocranium is invested by the **parasphenoids, palatines,** and **vomer.** The lower jaw is invested by a series of bones of which the most important is the **dentary.**

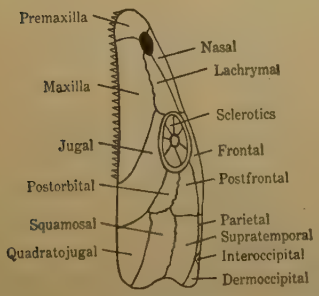

FIG. 116. — Cranial elements. Lateral view. Compare Figs. 111, 112.

The appendicular skeleton. — The simplest forms of appendages, the unpaired and paired fins of fish, contain a metameric

skeleton consisting of parallel cartilaginous rods, which are divided into proximal portions, **basalia,** embedded in the body, the distal portions, **radialia,** extending into the free appendages. The paired appendages of fish are paddle-like fins; in tetrapods they are jointed legs. In both, the skeleton is divided into a basal girdle and a free appendicular skeleton (Fig. 117).

The girdles. — The girdles are in the form of inverted arches, of which the pectoral girdle is united to the axial skeleton in fish and free in the tetrapods, while the pelvic girdle, usually free in fish, is united to the axial skeleton in the tetrapods. Each arch typically consists of three portions. The dorsal one in the pectoral girdle is the **scapula;** in the pelvic girdle it is called the **ilium.** The two ventral elements of the pectoral girdle are the **precoracoid** (anterior) and the **coracoid** (posterior), while the corresponding elements of the pelvic girdle are the **pubis** and **ischium.** In the shoulder region, the **clavicle,** a derm bone, becomes associated with the pectoral girdle.

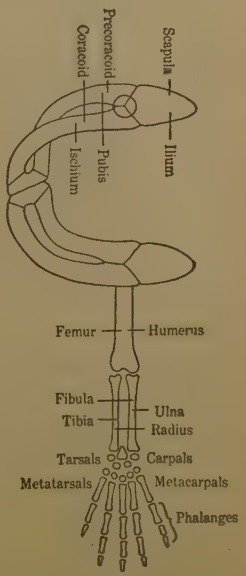

FIG. 117. — Diagram of tetrapod girdle and free appendicular skeleton. Pectoral elements of girdle to left, pelvic to right. Pectoral elements of appendage above, pelvic below. (After Kingsley, "Comparative Anatomy of the Vertebrates," copyright, 1926, by P. Blakiston's Son and Co., modified.)

The free appendages. — The pectoral and pelvic appendages are very similar. Each has three segments: proximal, intermediate, and distal. The proximal segment of the pectoral appendage contains one bone, the **humerus,** while the corresponding bone of the pelvic limb is called the **femur.** The intermediate portion of the pectoral limb possesses two bones, the **radius** and **ulna;** while the corresponding bones of the pelvic appendage are the **tibia** and **fibula.** The distal segment is divided into three regions of which the proximal portion contains nine or ten bones, the **carpalia** of the pectoral appendage, **tarsalia** of the pelvic. The intermediate

portion contains five **metacarpalia** or **metatarsalia,** respectively. The distal portion contains the free **phalanges** of the fingers or toes.

TABLE VII

Homologies of appendicular skeleton

Pectoral	General	Pelvic
	Girdle	
Scapula		Ilium
Procoracoid		Pubis
Coracoid		Ischium
	Free appendage	
Humerus		Femur
Radius		Tibia
Ulna		Fibula
Carpalia		Tarsalia
Metacarpalia		Metatarsalia
Phalanges (I–V)		Phalanges (I–V)

Origin of the appendicular skeleton. — All the bones of the appendicular skeleton, with the exception of the clavicle, are formed from a mesenchymal blastema in the limb buds by the appearance of centers of chondrification. The origin of this mesenchyme is probably from the somites, but the details of the process are still imperfectly understood.

The frog. — Nine vertebræ are formed, of which the first is known as the cervical vertebra, or atlas, the succeeding seven are the abdominal vertebræ, while the last is called the sacral vertebra as it is to this that the pelvic girdle is attached. No caudal vertebræ are formed, but three strips of cartilage enclose the notochord and form the primordium of the adult urostyle. Dorsal ribs are differentiated, but these remain rudimentary and fuse with the transverse processes of the vertebræ. The sternum arises from the fusion of two longitudinal bars of cartilage which never attain connection with the ribs. It persists anterior and posterior to the pectoral girdle.

The cartilage bones of the skull are the exoccipitals, proötics, stapes, ethmoids, and the pterygoquadrate (in part), articulare,

mentomeckelian, hyoid, and branchials. The derm bones are the fronto-parietal, nasals, premaxillæ, maxillæ, quadratojugals, squamosals, parasphenoid, palatines, vomers, and dentaries.

In the pectoral girdle develop the scapula, coracoid, and precoracoid, the last of which is replaced by the clavicle. In the pelvic girdle only the ilium and ischium ossify. Only four digits are present in the hand, the thumb (pollex) being absent.

The chick. — There are sixteen cervical vertebræ, of which the first is the atlas, and the second, which has appropriated the centrum of the first, is the axis; five thoracic vertebræ; about six lumbar vertebræ; two sacrals; and about fifteen caudals. The last thoracic, all lumbars, and sacrals and five caudals are fused to the pelvic girdle. The last four caudals are fused into a pygostyle. Dorsal ribs are formed by the cervical and the thoracic vertebræ. The sternum arises from two longitudinal bars of cartilage which unite in the median line. It is distinguished by the development of a large keel (carina) for the attachment of the pectoral muscles.

The cartilage bones of the skull are the basioccipital, exoccipitals and supraoccipitals; proötics, epiotics and opisthotics; basisphenoid, orbitosphenoids, and alisphenoids; the ethmoid; quadrate, articular, meckelian cartilage; stapes, hyoid, and branchils. The derm bones are the frontals, parietals, nasals, lachrymals, premaxillæ, maxillæ, jugals, quadratojugals, squamosals, pterygoids, palatines, parasphenoids, vomer, angular, supra-angular, opercular, and dentary.

The pectoral girdle develops a scapula and coracoid, together with a dermal clavicle. Ilium, ischium, and pubis ossify separately in the pelvic girdle. Five digits are preformed in the pectoral appendage; of these the first and fifth fail to develop further. Five also appear in the embryonic skeleton of the pelvic appendage; the fifth soon disappears, and the first is extremely short and develops no phalanges.

Man. — Seven cervical vertebræ, including the axis and atlas, twelve thoracic, five lumbar, five sacral, and four caudal vertebræ are formed. Of these, the sacral vertebræ are united to the pelvis, and the caudal vertebræ are frequently fused to form the coccyx. Primordia of ribs are formed by all vertebræ except those following the first caudal. Only the thoracic segments,

however, develop complete ribs. The sternum arises from two longitudinal primordia with which the first eight or nine ribs acquire cartilaginous connections.

The cartilage bones of the skull are the occipital (in part), the sphenoid, the ethmoid, the turbinates, temporals (in part), the stapes, malleus, incus, and hyoid. The malleus and incus are the representatives of the articular and quadrate. The derm bones are the occipital (in part), temporals (in part), frontal, parietals, lachrymals, nasals, vomer, maxillæ, zygomatics, palatines, and mandible, the last-named bone representing the fused dentaries. It is apparent that many of the bones of the human skull are the result of the fusion of separate centers of ossification which represent skull elements of the lower vertebrates. The second and third visceral arches contribute to the formation of the hyoid, the others to the laryngeal cartilage.

The pectoral girdle consists of the scapula, with which is fused the coracoid. There is no precoracoid, but a dermal clavicle is present. The centers of ossification that represent the pubis, ischium, and ilium fuse to form an innominate bone. The free appendages are pentadactyl. In conclusion, it should be mentioned that the adult condition of the human skeleton is not attained until the age of twenty-five.

G. THE MUSCLES

The musculature of the vertebrate is derived from mesenchyme (Fig. 118), of which the greater part originates from the myotomes and gives rise to **striated** muscle cells controlled by the central nervous system, the **skeletal musculature.** A portion, however, originates from splanchnic mesoderm and gives rise to **non-striated** (smooth) muscle cells found in the skin, surrounding the alimentary canal, blood vessels, and the urogenital organs. They are controlled by the autonomic nervous system (page 192), and make up the **visceral musculature.** Several exceptions to these general statements should be noted. The muscle cells of the heart are striated; the muscles derived from the visceral arches are both striated and controlled by the central nervous system, although derived from the epimere. It will be noted later that the muscles of the iris of the eye (page 204) and of the sweat glands (page 184) are apparently ectodermal in origin.

Dermal musculature. — In the skin are found striped muscles which are derived from skeletal musculature (see below) but which have lost their attachment to the skeleton. The dermal musculature is best developed in the amniota. The muscles of expression in man are dermal muscles supplied by the seventh cranial nerve (see Chapter VII).

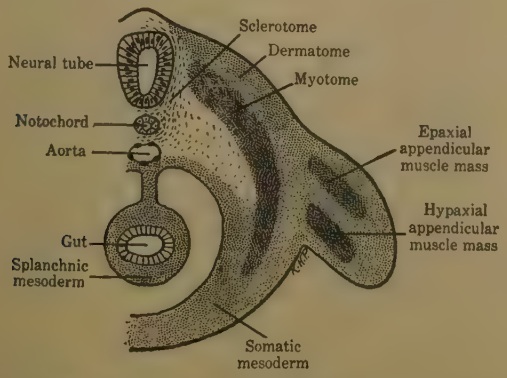

FIG. 118. — Diagram of cross section to show origin of vertebrate muscles. (After Corning.)

Axial musculature. — In this section are included all the muscles arising from the myotomes and attached to parts of the axial skeleton, which they move. They are originally metameric, but their later history is obscured by subsequent migration, fusion, splitting, budding, and degeneration. The **intercostals,** between the ribs, however, preserve their original metamerism, which in the others may be traced to some extent by the innervation, since the connection between a spinal nerve and the muscle mass it supplies is established early in organogeny and remains constant. Thus it can be shown that the musculature of the diaphragm, supplied by the phrenic nerve, arises from a cervical myotome.

Cranial muscles. — Like the cranium, the associated muscles are derived from different sources and consist of skeletal and visceral muscles. The muscles of the eyeball arise from the three cephalic myotomes (Fig. 119), of which the first supplies all the muscles of the eyeball except the **superior oblique,** derived from

the second myotome, and the **lateral rectus,** supplied by the third head myotome. These are innervated by the third, fourth, and sixth cranial nerves, respectively. The tongue musculature is derived from the myotomes associated with the occipital vertebræ and supplied by the twelfth cranial nerve. The muscles of mastication, the facial muscle, and the laryngeal muscles, together with those of the ear bones, arise from the visceral arches (Fig. 120), and are supplied by cranial nerves V, VII, IX, X, and XI (see Chapter VII).

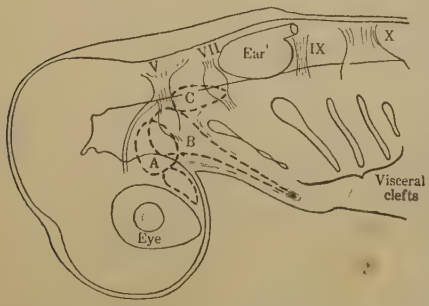

FIG. 119. — Head of embryo Acanthias, showing preotic somites. A, B, C, head somites. V, VII, IX, X, cranial nerves. (After Kingsley, "Comparative Anatomy of Vertebrates," copyright, 1926, by P. Blakiston's Son and Co., modified.)

Appendicular muscles. — In the anamniotes, these muscles arise from the myotomes; among the amniotes, their origin is doubtful, as the limb bud develops as an undifferentiated mass of

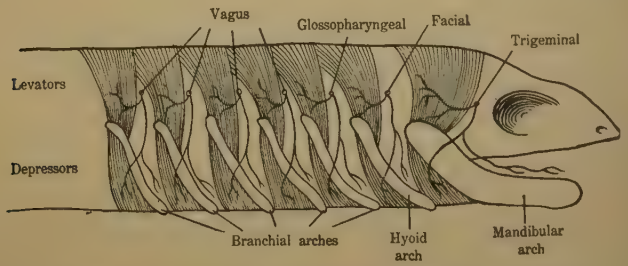

FIG. 120. — Diagram of primitive branchiomeric (visceral) musculature. (After Wilder, "History of the Human Body," copyright, 1923, by Henry Holt and Co., modified.)

mesenchyme surrounded by ectoderm. In this blastemal mass, muscles and bones are laid down, the differentiation proceeding from the proximal toward the distal end. The pectoral muscles differentiate before those of the pelvic appendage. The appen-

dicular muscles are found in antagonistic groups: **protractors,** which move the limb forward; and **retractors,** which have the opposite effect; **levators,** which raise the limb; and **depressors,** which contract in the opposite direction. Like the axial muscles, these have become highly modified and specialized among the tetrapoda (Fig. 121).

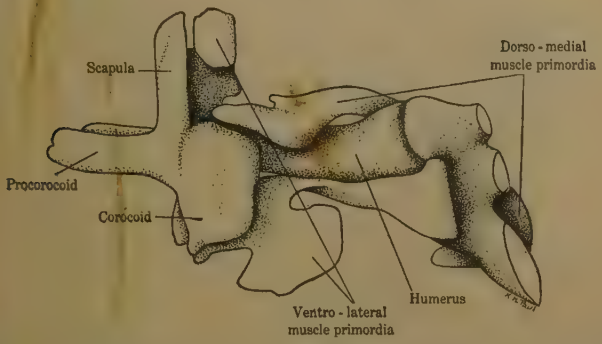

FIG. 121. — Pectoral musculature in a 17 mm. Necturus. (From a wax reconstruction by H. F. DeBruine.)

Visceral muscles. — Under this head are included the muscles lining the alimentary tract, lungs, vascular organs, and urogenital system. All arise in the mesoderm which surrounds the endothelial lining of the organs concerned. The muscle cells of the heart arise as smooth muscle cells which become striated in later development. It is interesting in this connection that the smooth muscle cells of the bladder of the dog have been transformed into what are apparently striate muscles when this organ is made to pulsate rhythmically by continued irrigation.

Summary. — The following structures are derived from mesoderm:

A. The notochord

B. The mesoderm proper

I. The hypomere

Cœlom

Pericardial cavity
Pleural cavity
Peritoneal cavity

Mesenteries

Dorsal mesentery
Ventral mesentery
Mesocardia
Mesohepares

II. The mesomere

Nephroi

Pronephros and duct
Mesonephros and duct
Metanephros and duct

Genitalia

Gonads
Gonoducts
Wolffian (mesonephric) duct
Müllerian (oviducal) duct
External genitalia

Adrenal glands

Interrenals
(Suprarenals from ectoderm)

C. The mesenchyme

III. (Principally from splanchnic mesoderm)

The blood corpuscles

Erythrocytes
Leucocytes

Blood plasma

Blood vessels

Heart
Arteries
Veins
Splanchnic
Somatic

The lymphatics

IV. (Principally from the epimere)
- Skeleton
  - dermal
  - axial
  - cranial
    - chondrocranium
      - neurocranium
      - splanchnocranium (from splanchnic mesoderm)
    - dermocranium
  - appendicular
- Musculature
  - dermal
  - axial
  - cranial
  - appendicular
  - visceral (from splanchnic mesoderm)

REFERENCES

Brachet, A. 1921. Traité d'Embryologie des Vertébrés, Part II, Bk. 1, Chap. 2; Bk. 2, Chaps. 1–4.
Graham-Kerr, J. 1919. Textbook of Embryology, Chaps. 4–6.
Hertwig, O. 1906. Handbuch, Vol. 1, Chap. 5; Vol. 3, Chaps. 1–7.
Jenkinson, J. W. 1913. Vertebrate Embryology, Chap. 7.
Keibel and Mall. 1910–1912. Human Embryology, Chaps. 11–13, 15, 18 and 19.
Kellicott, W. E. 1913. Chordate Development.
Kingsley, J. S. 1926. Comparative Anatomy of Vertebrates, 3rd Ed.
Kingsley, J. S. 1925. The Vertebrate Skeleton.
Lillie, F. R. 1919. The Development of the Chick, 2nd Ed.
McMurrich, J. P. 1923. The Development of the Human Body, 7th Ed.
Vialleton, L. 1924. Membres et Ceintures des Vertébrés Tétrapodes.

CHAPTER VII

ECTODERMAL DERIVATIVES

The ectoderm, being the external germ layer, gives rise to the outer layer of the skin, the epidermis, which continues into all of the openings of the body. Of these, the development of the mouth, the cloaca and its derivatives, and the visceral clefts has been discussed. There remain for consideration the openings of the nostrils, the chamber of the eye, and the external auditory meatus. These will be taken up in connection with the sense organs, which, with the nervous system, form in development a sensory-nervous complex, withdrawn beneath the surface of the body.

A. THE INTEGUMENT

The integument consists of two parts, the ectodermal **epidermis,** and the mesodermal **dermis.** The epidermis soon delaminates into two layers, the deeper **germinativum,** from which new strata are proliferated towards the exterior, and an outer **periderm** or embryonic skin (Fig. 122). Beneath the periderm, the outer cells of the germinativum are transformed into a horny layer, the **corneum.** The underlying dermis is essentially a supporting layer of mesenchyme cells derived largely from the outer side of the myotome, a region which is sometimes known as the dermatome. In the dermis are formed blood vessels, connective tissue, bone, and muscle. The **bony scales** of fish are dermal in origin.

Derivatives of the corneum. — In the amniota the horny layer of the epidermis is frequently fragmented to form **horny scales** (Fig. 123*A*), such as those of reptiles, or those found on the legs of birds, or the tails of rats, etc. Among the birds, scales are largely replaced by **feathers** which originate in much the same manner as scales. The epidermal plate, however, grows down like a cup to enclose a core of dermal origin (Fig. 123*B*). The epidermal sheath gives rise to the quill and barbs, while the core

gives rise to the pulp, by means of which nutriment is supplied to the developing feather. Among the mammals, **hair** arises in a

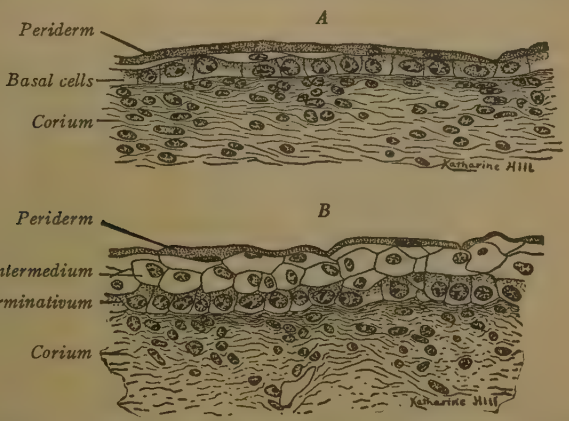

FIG. 122. — Sections of the skin of the human fœtus. A, neck. B, chin. (After Prentiss. From Arey, "Developmental Anatomy," copyright, 1924, by W. B. Saunders Co.)

very similar fashion. An epidermal plate grows down into the dermis to form the hair bulb, the proximal end of which invaginates to receive a mesodermal core, the hair papilla, while

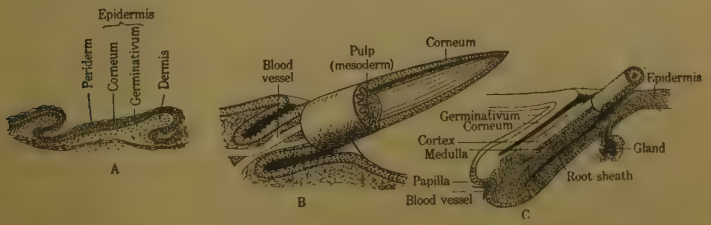

FIG. 123. — Diagrams to show development of A, scale; B, feather; and C, hair. (After Kingsley, "Comparative Anatomy of Vertebrates," copyright, 1926, by P. Blakiston's Son and Co., modified.)

around the whole is a mesodermal hair sheath (Fig. 123*C*). The hair papilla, however, does not grow out into the center of the hair as does the pulp of the feather. Claws, nails, and hoofs arise from the union of two epidermal primordia like those of scales, a dorsal **unguis** and a ventral **subunguis** (Fig. 124).

Derivatives of the germinativum. — The germinativum, in addition to producing the more superficial layers of the epidermis, gives rise to the glands of the skin (Fig. 125). Among the an-

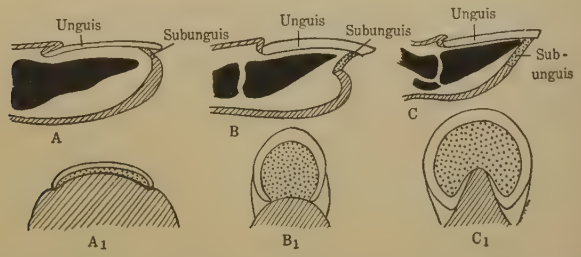

FIG. 124. — Diagrams of A, nail; B, claw; and C, hoof. (After Kingsley, "Comparative Anatomy of Vertebrates," copyright, 1926, by P. Blakiston's Son and Co., modified.)

amniotes, these glands are usually unicellular and produce the **mucus** which serves to diminish the friction of the skin against the water while swimming. Unicellular glands frequently aggregate to produce multicellular glands, such as the **flask glands** and **cement glands** of the anamniotes, or the **sebaceous** (oil) and **sudoriparous** (sweat) **glands** of the mammals. The **mammary glands** of mammals are modified sudoriparous glands secreting the milk by which the new born are nourished through infancy.

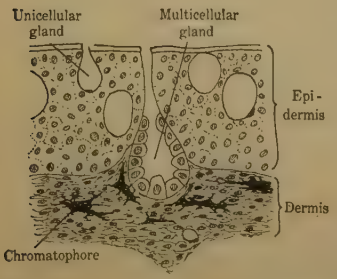

FIG. 125. — Section of Protopterus skin. (After Kingsley, "Comparative Anatomy of Vertebrates," copyright, 1926, by P. Blakiston's Son and Co., modified.)

Derivatives of the dermis. — Two types of pigmentation are to be distinguished in the integument. The first is produced by pigment secreted in the ectodermal epidermis, i.e., the melanin, of the frog tadpole. The second is produced by **chromatophores,** which are mesenchyme cells of the dermis. These secrete pigment granules and move toward the light to form a layer immediately below the epidermis, some even wandering into the epidermis itself.

The frog. — The ectoderm of the frog embryo is ciliated at 6 mm. body length and remains so until the length of 20 mm. is attained, when the cilia disappear except on the tail which remains ciliated until metamorphosis. The jaws and oral combs of the tadpole are derivatives of the corneum and consist of rows of horny denticles forming replacement series. The cement gland, or "**sucker,**" is a multicellular mucous gland derived from the germinativum and elevated by the elongation of its gland cells. It arises as a crescentic groove posterior and ventral to the point where the stomodæum will appear, then becomes V-shaped, and finally divides by the degeneration of the middle portion. The cement gland atrophies soon after the opening of the mouth. The pigmentation of the skin is derived from two sources, the melanin of the egg which is distributed to the epidermis, and the mesenchymal chromatophores which develop in the dermis.

The chick. — The scales on the legs are typical reptilian scales and are derived from the corneum; they sometimes bear feathers in the young bird and so form a transition between scales and the characteristic avian feathers. The claws arise in the corneum from two primordia, a dorsal "**claw-plate**" and a softer "**claw-sole.**" To prevent the sharp claws tearing the embryonic membranes, the concavity of the claw is filled with a pad known as the **neonychium,** derived from the corneum, which is lost after hatching. The beak arises from the corneum around the upper and lower margins of the jaws. The **egg tooth** is a horny prominence on the dorsal side of the upper jaw, appearing on the sixth day of incubation but not taking on its ultimate shape until the fourteenth. It serves to break the shell and is lost after hatching.

Man. — The nails arise from **nail-plates** and **sole-plates,** of which the latter are rudimentary structures. They are covered during fœtal life by the **eponychium,** consisting of the periderm and outer layers of the corneum. The hairs are arranged in patterns which have been interpreted as reminiscences of the ancestral scales. The first growth of hair is called the **lanugo;** it is cast off, except over the face, soon after birth. The **mammary glands** arise from two longitudinal thickenings of the epidermis, known as the **milk ridge.** In later development the gland resembles an aggregation of sudoriparous glands.

B. THE NERVOUS SYSTEM

Although the nervous system and sense organs arise together and remain in functional continuity, it has become customary to distinguish the **sense organs** (**receptors**) from the **nerves** (**transmittors**) by which stimuli are passed on to the muscles or glands (**effectors**). Both the nervous system and the sense organs arise from specialized regions of the dorsal ectoderm, known respectively as the **neural plate** and the **sense plates** (**placodes**). These represent an inward growth from the germinativum as opposed to the outward growth which produces the epidermis. In the frog this division is clearly indicated by a line of cleavage between the outer epidermal ectoderm and the inner nervous ectoderm. Both the neural plate and the sensory placodes withdraw from the surface and become subepidermal by a process of invagination. In this connection it is interesting to note that, of the three sensory placodes, olfactory, optic, and otic, the optic placode is incorporated and invaginates with the neural plate *so that when the retina of the eye develops, it does so from the brain.*

The neural tube. — The neural plate is an elongate structure, extending from the blastopore to the head region. Local growth results in the incurving of this plate to produce a **neural groove** with conspicuous lips, the **neural folds.** As this growth continues the groove sinks inward and the lips meet above it, thus converting the groove into a neural tube, which breaks away from the overlying epidermis and sinks into the interior. The cells at the margin of the neural plate form, at each dorso-lateral angle of the neural tube, a bar known as the **neural crest,** which subsequently segments into the **ganglia.**

The neurones. — The inner lining of the neural tube, corresponding to the outer layer of the neural plate, is called the **ependyma.** This is the center of cell proliferation (Fig. 128). Two types of cells are formed: the supporting cells, or **spongioblasts;** and the embryonic nerve cells, or **neuroblasts.** The neuroblasts migrate out of the ependyma and form an intermediate **mantle layer** in which they become transformed into neurones. These nerve elements have a prolongation at one end known as the **axon** or nerve fiber, while at the other are branched projections called **dendrites.** The axons grow out

from the mantle layer into the outer layer of the cord, known as the **marginal layer,** where they secrete the **medullary sheaths** which act as insulating coats. Not all axons become medullated. Similar changes take place in the ganglia, whereby neurones and supporting cells are differentiated.

Types of neurones. — We may distinguish four types of neurones (Fig. 126), as follows: (1) **Afferent neurones** arising in the ganglia and sending their axons to the dorsal region of the neural tube. These convey excitations from the sensory receptors to the neural tube. Two sub-types are distinguished: (*a*) the **somatic sensory neurones,** conveying excitations from the exterior; and (*b*) **splanchnic sensory neurones,** conveying excitations from the viscera. (2) **Efferent neurones,** with their bodies in the ventral region of the neural tube, sending their axons to effectors (muscles or glands). Two sub-types are recognized: (*a*) **somatic motor** and (*b*) **splanchnic motor.** These afferent and efferent neurones form the peripheral nervous system. (3) The **intersegmental neurones** have their bodies in the ventral portion of the neural tube and their axons are usually directed to its posterior end. They serve to connect efferent neurones in the different segments of the body. (4) The **suprasegmental neurones**

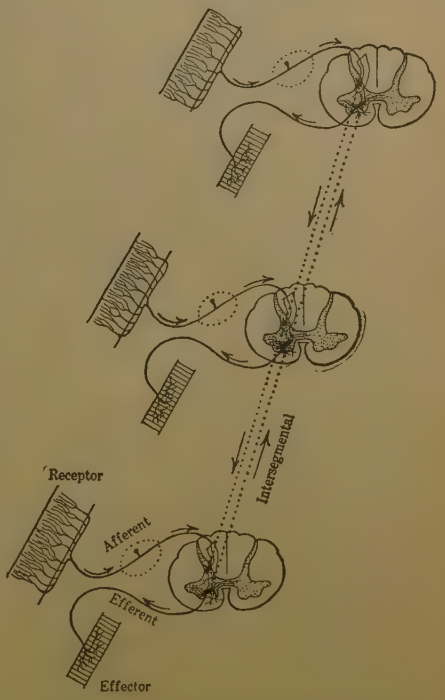

FIG. 126. — Diagram showing neurones of three types.

have their bodies usually in the dorsal portion of the neural tube and their axons are directed toward the anterior end of the tube, i.e., the brain. They serve to convey afferent excitations toward the brain and in that organ give rise to the great **suprasegmental centers.** The axons of these last two types of neurones form the **descending** and **ascending bundles** of the brain and cord.

The spinal cord. — The **spinal cord,** or neural tube exclusive of the brain, retains its primitive characteristics. The cavity, or neurocœle, persists as the **central canal.** Between each pair of vertebræ the afferent and efferent neurones form a pair of **spinal nerves** which run out into the myotomes and hence have a metamerism equivalent to that of the myotomes, an important point in considering the homologies of the muscles. In the region of the pectoral and pelvic appendages, several of the segmental nerves combine to form the **brachial** and the **sacral plexus,** respectively. The cord becomes surrounded by an envelope of mesenchyme known as the **meninx,** which in the higher vertebrates becomes divided into an inner **pia mater** and an outer **dura mater.** The development of the nerves will be taken up in a later section.

The brain. — While the cord is largely composed of afferent, efferent, and intersegmental neurones, by which certain reflex actions are directed, the anterior end of the neural tube enlarges and differentiates into the complex brain (Fig. 127). Here arise several suprasegmental centers in which correlation of the impulses, received mainly from the major sense organs, nose, eye, and ear, are accomplished. The brain may be divided into two major regions: the **archencephalon,** or **prechordal brain;** and the **deutencephalon,** or **epichordal brain.** With continued local growth, the archencephalon grows down in front of the notochord, thus forming the first or **cranial flexure.** At the same time, three dilations appear: the **prosencephalon** from the archencephalon; the **mesencephalon** at the point of the flexure; and the **rhombencephalon** from the deutencephalon. It is convenient to associate the future history of the prosencephalon with the olfactory organ, the mesencephalon with the optic organ, and the rhombencephalon with the otic organ or, better, the **acustico-lateralis system,** of which the ear is a part.

The prosencephalon. — The later history of the prosencephalon is complicated by the fact that the optic placode is included in the neural tube at this point. Accordingly, we find the prosencephalon dividing into an anterior **telencephalon** and a posterior **diencephalon.**

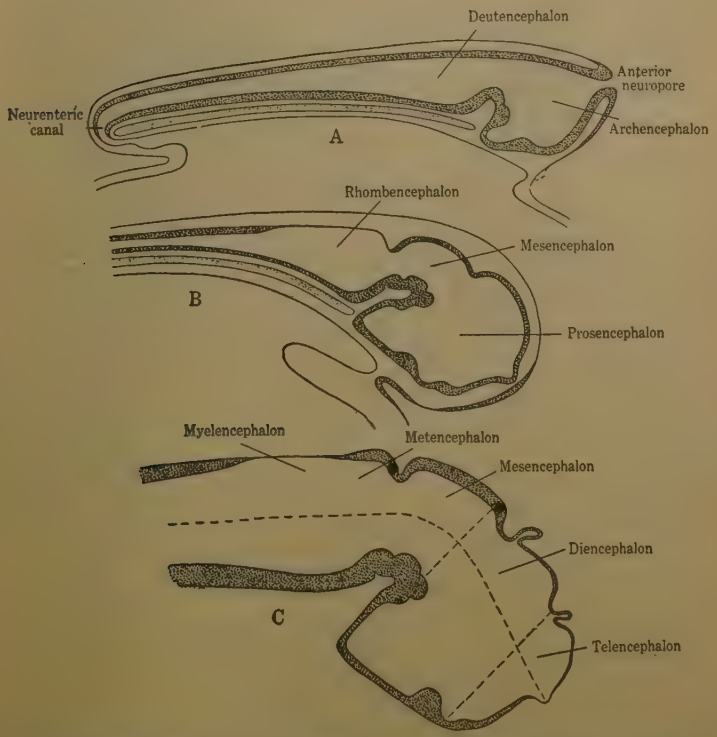

FIG. 127. — Sagittal sections of embryonic brains. A, primary division of the brain. B, the three primary brain vesicles. C, the five definitive vesicles. (After von Kupffer.)

The telencephalon. — The anterior part of the telencephalon becomes the **rhinencephalon,** which receives the afferent neurones from the nose. The roof becomes the **cerebrum,** first the suprasegmental center associated with the nose, later the most complex and important **center of association.** From the floor arises

the **optic part** of the **hypothalamus.** There are two cavities, or **telocœles.**

The diencephalon. — The roof of the diencephalon gives rise to the **thalamus** in front, and the **metathalamus** behind, while from the latter springs a dorsal diverticulum, the **epithalamus.** This structure, often known as the **epiphysis,** gives rise to a structure very much resembling an unpaired eye in early embryonic life; this later becomes the **pineal gland** of the adult, one of the so-called endocrine glands. The eyes take their origin from the side of the diencephalon. The floor of the diencephalon gives rise to a ventral diverticulum — the **infundibulum,** which grows downward to meet the advancing hypophysis from the stomodæum (see page 120). The two later fuse to form the **pituitary gland,** another of the endocrine series. Behind the infundibulum, the floor of the diencephalon forms the **mammillary part** of the **hypothalamus.** It is evident that the **thalamencephalon,** often used as a synonym of the diencephalon, differs from it by the inclusion of the optic part of the hypothalamus, which is derived from the telencephalon although indistinguishable from the mammillary part of the hypothalamus in the adult. The thalami contain nuclei which receive afferent impulses from the optic, general sensory, and acoustic organs, and transmit impulses to and from the suprasegmental centers. The cavity of the diencephalon persists as the **diacœle.**

The mesencephalon. — The roof of the mesencephalon gives rise to the **corpora bigemina** (**quadrigemina** in mammals) or optic lobes, the second of the suprasegmental centers, which receive afferent impulses from the eyes transmitted through the diencephalon. The floor of the mesencephalon is the anterior portion of the **brain stem,** from which the motor neurones of the cranial nerves depart. The third and fourth cranial nerves originate from the mesencephalon. Its cavity is the **mesocœle** (or **aqueduct**).

The rhombencephalon. — The hind-brain, like the fore-brain, is divided into two regions, **metencephalon** and **myelencephalon,** respectively.

The metencephalon. — The roof of the metencephalon gives rise to the **cerebellum,** the suprasegmental center originally connected with the acustico-lateralis system and later associated

with the otic organ and the sense of equilibrium. The floor of the metencephalon is part of the brain stem, and from it arises the **pons,** a bundle of axons connecting the two sides of the cerebellum. The cavity is the **metacœle.**

The myelencephalon. — The roof of the myelencephalon is covered by a thin roof plate, the **choroid plexus.** Its floor forms the posterior portion of the brain stem. The cranial nerves, from VI to XII inclusive, depart from this portion of the stem, which merges imperceptibly into the spinal cord. Its cavity, hardly distinguishable from that of the metencephalon, is called the **myelocœle.**

The spinal nerves. — The nerves are segmentally arranged bundles of afferent and efferent neurones which originally con-

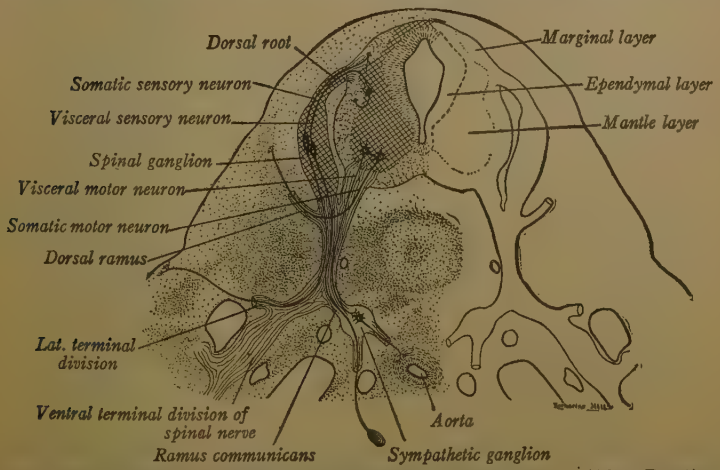

FIG. 128. — Diagram of neurone components in a spinal nerve. (After Prentiss. From Arey, "Developmental Anatomy," copyright, 1924, by W. B. Saunders and Co.)

nected the myotomes and their derivatives with the central nervous system. The afferent neurones arise in the ganglia, the efferent in the floor of the spinal cord. Accordingly, a typical spinal nerve has two **roots** in the cord: a dorsal afferent root uniting with the ganglion; and a ventral efferent root which unites with the dorsal root after the other has attached itself to the ganglion (Fig. 128). The **nerve trunk** then divides into

branches, each containing afferent and efferent neurones, which are called **rami** and supply the body wall, although one (the **ramus intercommunicans**) connects with a sympathetic ganglion, derived from a spinal ganglion, through which the splanchnic afferent and efferent neurones serve the viscera.

The autonomic nerves. — The brain, cord, and cerebro-spinal nerves are grouped by anatomists as the **central nervous system.** Associated with this is the **autonomic nervous system,** consisting of nerves and ganglia and controlling the smooth muscles of the viscera and blood vessels, and some glands. This system arises from the neural plate, like the central nervous system, but from the lateral margins which become the neural crests. At the time when the neural crests are dividing into the cerebro-spinal ganglia, some of the cells migrate inward toward the dorsal aorta, where they aggregate and multiply to form the **chain ganglia.** The chain ganglia on each side become connected by fore and aft extensions which form the **sympathetic trunks.** They retain a connection with the cerebrospinal ganglia by means of the rami intercommunicantes, and send out nerves along the principal blood vessels. From the chain ganglia, by secondary and tertiary migrations, arise the **prevertebral** and **visceral ganglia.** In the head the four sympathetic ganglia (**ciliary, sphenopalatine, otic,** and **submaxillary**) arise from the semilunar ganglion of the fifth cranial nerve, and later acquire connections with the chain ganglia (Fig. 129).

The cranial nerves. — The cranial nerves, or nerves of the head regions, contain not only splanchnic and somatic afferent and efferent neurones comparable to those of the spinal nerves, but also special afferent neurones from the nose, eye, and acusticolateralis system. There are ten cranial nerves in the anamniotes, twelve in the amniotes (Figs. 130, 131). To these should be added in all cases the **nervus terminalis,** unknown when the cranial nerves were first classified.

A. Nervus terminalis, a ganglionated nerve from the organ of Jacobsen entering the rhinencephalon with functions unknown.

I. Nervus olfactorius, a non-ganglionated sensory nerve from the olfactory sensory region of the nose to the rhinencephalon.

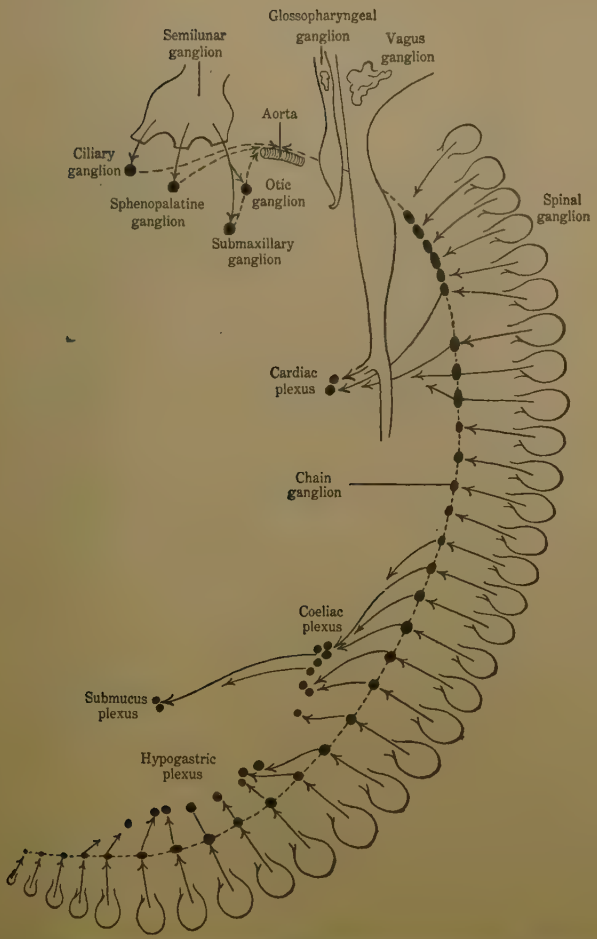

FIG. 129. — Diagram showing migrations of the autonomic ganglia. (After Streeter, modified.)

II. Nervus opticus, a non-ganglionated sensory nerve from the retina of the eye to the floor of the diencephalon where the fibers from the two eyes cross (**optic chiasma**) and enter the brain, running to the optic lobes, on the opposite side of the brain to that on which the eye is located.

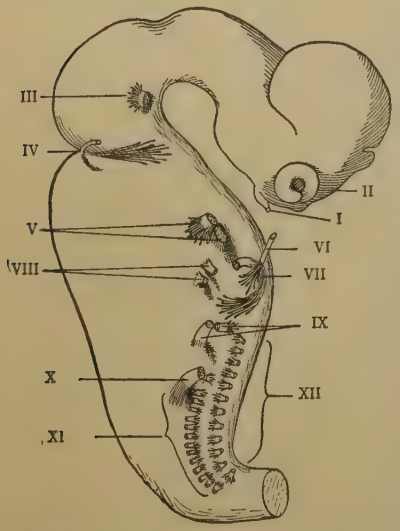

Fig. 130. — Diagram to show cranial nerves of human fœtus. (After His.)

III. Nervus oculomotorius, a motor nerve, somatic with some sensory elements, from the floor of the mesencephalon to all muscles of the eyeball except the superior oblique and the lateral rectus.

IV. Nervus trochlearis, a motor nerve, somatic with some sensory elements, from the roof of the mid-brain to the superior oblique muscle of the eyeball.

V. Nervus trigeminalis, a mixed nerve. Its somatic sensory neurones arise in the **semilunar ganglion,** the motor elements in the floor of the myelencephalon. The sensory neurones are somatic (general cutaneous). The motor neurones supply the jaws (mandibular arch).

VI. Nervus abducens, a somatic motor nerve with some sensory elements, arising from the myelencephalon and supplying the external rectus muscle of the eyeball.

VII. Nervus facialis, a mixed nerve. The afferent neurones arise in the **geniculate ganglion** and are splanchnic in nature, originally supplying the hyoid arch, the tongue in mammals. In the anamniota, an associated ganglion gives rise to a **lateralis** branch with afferent components. The efferent neurones supply the hyoid arch in the lower vertebrates and the facial region in the amniota. The rami of the fifth and seventh nerves are closely associated.

VIII. Nervus acusticus, a ganglionated sensory nerve arising from the **acoustic ganglion** and bearing afferent neurones from the ear. In higher vertebrates it becomes differentiated into the **vestibular** and **cochlear nerves,** each with its own ganglion produced by the division of the acoustic ganglion.

IX. Nervus glossopharyngeus, a mixed nerve. The afferent neurones arise in the **petrosal** and the **superior** ganglion and are principally splanchnic. They divide into a prebranchial branch running into the hyoid arch and a postbranchial branch into the first branchial arch. The efferent components are principally found in the postbranchial branch.

X. Nervus vagus, a mixed nerve arising by the fusion of several primitive cranial nerves, which supplied the arches with afferent (from the **jugular** ganglion) and efferent neurones. In addition, the vagus gives off a **visceral** branch to the stomach, lungs, etc., and in the anamniota a **lateralis** branch to the lateral line organs of the trunk.

XI. Nervus accessorius, a motor nerve which innervates the muscles of the shoulder girdle and is found only in the amniota. A ganglion (**of Froriep**) disappears before the embryo becomes adult.

XII. Nervus hypoglossalis, also a motor nerve, which innervates the tongue in the amniota. In the anamnia the tongue is innervated by so-called " occipital " nerves which possibly are the fore-runners of the hypoglossal, prior to the appropriation of the occipital region by the head.

Metamerism of the nervous system. — The metameric arrangement of the nerves, like that of the segmental arteries, is purely secondary and dependent upon the primary metamerism of the mesoderm. The nerves, however, are more conservative than the vascular organs or myotomic derivatives. For example, the diaphragm of mammals is supplied by muscles from one of the cervical myotomes, and the innervation of the diaphragm (phrenic nerve) still arises from the cervical region. Many attempts have been made to reconstruct the metamerism of the head by a study of the cranial nerves, following **Bell's law:** that every original cranial nerve has, like a spinal nerve, a dorsal sensory and ventral motor root.

This problem has been complicated by the fact that in the

head there are two types of metamerism, (1) **primary** as indicated by the head myotomes in the chondrichthyes, and (2) secondary or **branchiomeric,** as indicated by the visceral arches (Fig. 131). Accordingly, there are two types of musculature, (1) somatic as represented by the muscles of the eyeball, and (2) splanchnic as represented by the muscles of the jaws and visceral arches. Two types of efferent neurones, therefore, are present, (1) somatic and (2) splanchnic. The splanchnic motor neurones of the cranial nerves differ from those of the trunk, however, in that no sympathetic neurones intervene between them and the muscles which they supply. There are altogether three sets of afferent

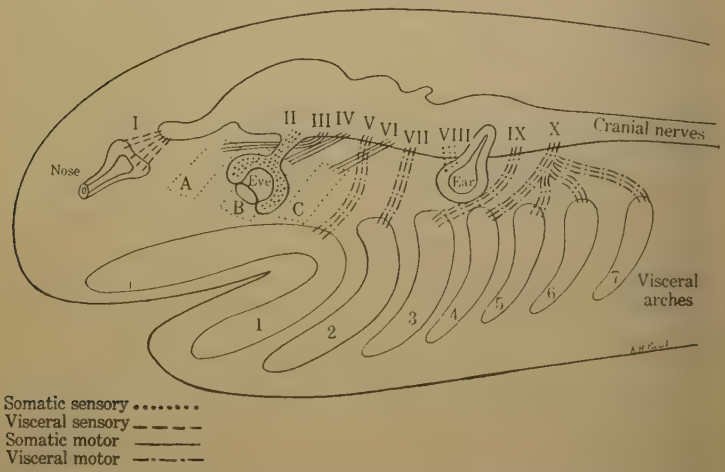

FIG. 131. — Diagram showing relationships between cranial nerves and parts supplied. A, B, C, head somites. Arabic numerals, visceral arches. Roman numerals, nerves.

neurones: (1) the general sensory or cutaneous, which correspond to the somatic sensory neurones of the trunk; (2) splanchnic sensory, which correspond to those of the trunk; and (3) lateralis, belonging to the lateral line system with which no homologous structure has been identified in the trunk. The cranial nerves are evidently not serially homologous, as can be seen from the following table of components.

TABLE VIII

Neurone components of cranial nerves and functions

Nerve	Afferent Somatic	Afferent Splanchnic	Efferent Somatic	Efferent Splanchnic
I		Smell		
II	Vision			
III			Movement of eyeball	
IV			Movement of eyeball	
V	General cutaneous			Movement of jaw
VI			Movement of eyeball	
VII		Taste		Hyoid and facial movement and salivation
VIII	Hearing and equilibration			
IX		Taste and pharyngeal sensation		Salivation, pharyngeal movement
X		Visceral sensation		Movement of viscera and pharynx
XI				Movement of pharynx and shoulder
XII			Movement of tongue	

Finally, we must mention the **neuromeres** which have been reported in various vertebrate embryos. These are formed by constrictions in the cranial portion of the neural tube and inter-

preted by some authors as the remains of a neural metamerism. They seem in many forms to correspond with the cranial nerves and more probably represent areas of local growth prior to the outgrowth of the nerves themselves.

The general problem of the metamerism of the head still awaits solution. The latest summary, that of Brachet, indicates the probable number of segments in the primitive head as six. Three of these are ephemeral, and their somites give rise to mesenchyme. The three posterior segments are associated with the first three visceral clefts bounded by the first four arches, each of which has its own cranial nerve: the trigeminal of the mandibular arch; the facial of the hyoid; the glossopharyngeal of the first branchial; the vagus of the second branchial arch. According to this interpretation, the posterior clefts and arches are reduplications supplied by new branches of the vagus, while the accessory and hypoglossal are secondarily acquired spinal nerves.

The frog (*See also Chapter VIII*). — The prechordal and epichordal divisions of the brain are demarcated by the notochord, but the division into the three primary vesicles is but slightly indicated. The brain of the frog never develops neuromeres. The optic lobes are corpora bigemina. The division into myelencephalon and metencephalon is incomplete, and no pons is formed. There are forty pairs of spinal nerves in the tadpole, reduced to ten in the adult. There are but ten of the cranial nerves (XI and XII not included). The sympathetic ganglia originate from the cranial and spinal ganglia by the emigration of ganglion cells.

The chick (*See also Chapter IX*). — The divisions of the brain into the three primary and five secondary vesicles is well marked. Eleven neuromeres are formed, of which three are found in the prosencephalon, two in the mesencephalon, the remainder in the rhombencephalon. Three flexures are formed, cranial at the junction of archencephalon and deutencephalon, pontine in the floor of the myelencephalon and spinal cord. A pons is formed. There are fifty pairs of nerves developed in the chick of eight days (Lillie), of which thirty-eight are spinal and twelve cranial, including the eleventh and twelfth which are not incorporated in the head of the frog.

Man (*See also Chapter X*). — The particular feature of importance in the development of the human brain is the great

increase in size and complexity of the cerebral hemispheres of the telencephalon. The optic lobes are quadripartite, corpora quadrigemina, of which the two anterior lobes are especially associated with vision, the two posterior with hearing.

C. THE SENSE ORGANS

The nervous system receives not only stimuli from outside the body but also from within, such as those concerning the tension of the muscles. For the reception of stimuli, special organs — the sense organs — are developed. Of these the major sense organs are the **eyes,** the **ears,** and the **nose.** In addition, it must be remembered that the entire skin functions as a sense organ by means of special receptors and that stimuli are received from the viscera and other internal structures by means of **free nerve terminations.**

Of the major sense organs, the eye is most specialized in its mode of development. It is responsive to photic stimuli. The nose represents a concentration of chemical sense receptors, more highly developed than the scattered taste buds of the head, which are confined in adult mammals to the cavity of the mouth. The ear, responsive to slower vibrations (pressure, sound) in the surrounding medium, is a specialized portion of the acusticolateralis system. The lateral line system is highly developed in the aquatic anamniotes, vestigial or absent in the amniotes. The ear, on the other hand, is more highly developed in the amniotes.

The nose. — The nose arises as a pair of local thickenings of the sense plate at the anterior end of the head (Fig. 132). These thickenings are hereafter known as the **olfactory placodes.** Later they invaginate to form the **olfactory pits,** which persist as the nose of all fish except the air-breathing dipnoi. Here also should be noted the fact that the cyclostomes are peculiar in the possession of a single median nasal pit. Among the tetrapods the nasal pits elongate to become **olfactory grooves,** the anterior ends of which become connected with the developing mouth into which they are carried. The original anterior ends of the olfactory pits, therefore, come to lie at the posterior end of the mouth and open into the pharynx as the **internal nares,** while the original posterior ends become the **external nares** (Fig. 132*E*). The nasal cavity is later separated from the oral cavity by the ingrowth of

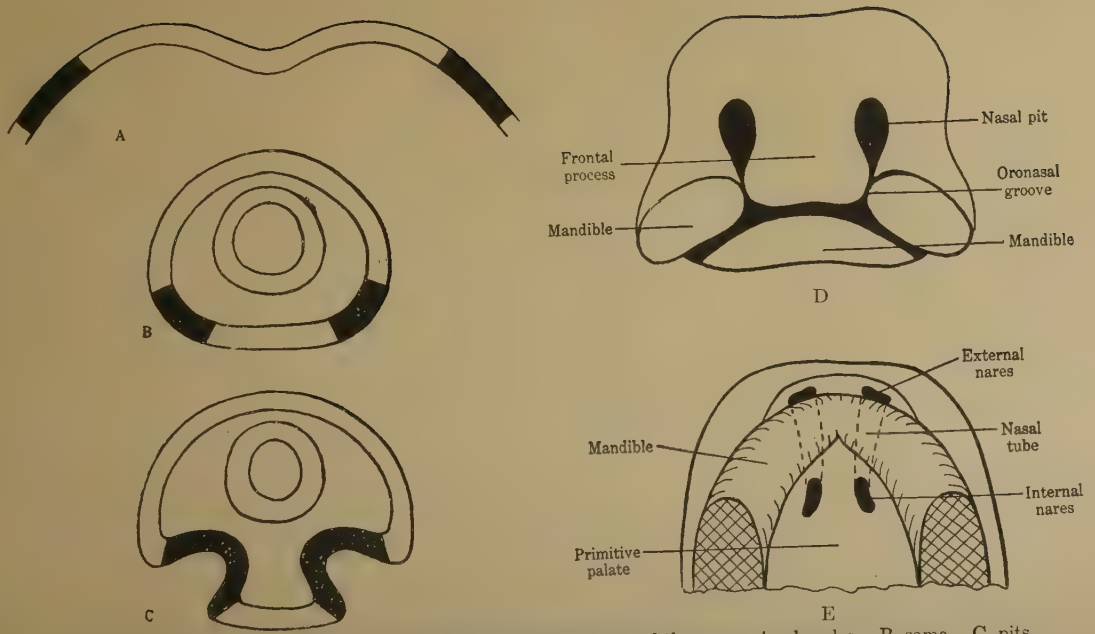

FIG. 132. — Diagrams to show early stages in the development of the nose. A, placodes. B, same. C, pits. D, nasal grooves. E, nasal tubes.

the maxillary, palatine, and pterygoid bones, which form the **hard palate** (Fig. 133). **Jacobsen's organ** arises as a pocket of the olfactory epithelium. Its function is unknown. The olfactory epithelium contains ciliated cells connected to the rhinencephalon by means of the first cranial nerve, which is peculiar in that its neurones run directly to the brain without the interposi-

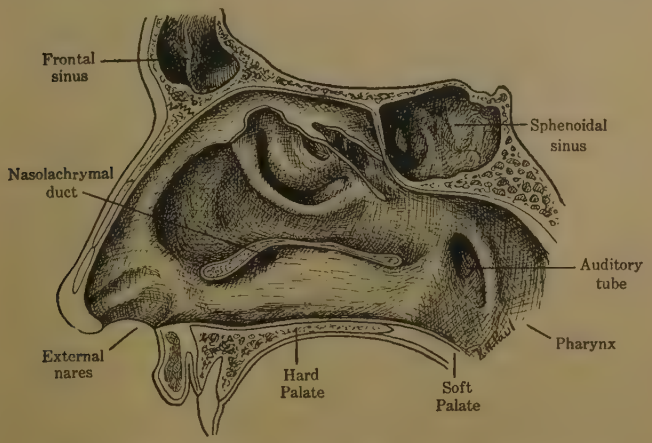

FIG. 133. — Sagittal section of human nose. (After Howden, modified.)

tion of ganglion cells. Jacobsen's organ receives a branch of the trigeminal nerve.

The eye. — The **optic placodes** are incorporated into the neural plate, where they can be distinguished as lateral thickenings of the margin at points which will later be included in the diencephalon (Fig. 134). When the tube is formed, the relation of the sensory epithelial cells to the exterior is, of course, reversed. The optic placodes "invaginate" but, owing to their relation to the neural tube, the result is an apparent "evagination" from the tube towards the exterior. This "evagination" produces the **optic lobes** and later, by constriction, the proximal **optic stalks** and distal **optic vesicles.** At the point where the optic vesicle touches the ectoderm, two reactions take place: (1) a local thickening of the ectoderm called the **lens placode,** from which the lens of the eye develops; and (2) an invagination of the optic vesicle whereby this vesicle is transformed into a

two-layered **optic cup.** This invagination continues into the optic stalk to produce a groove called the **chorioid fissure.**

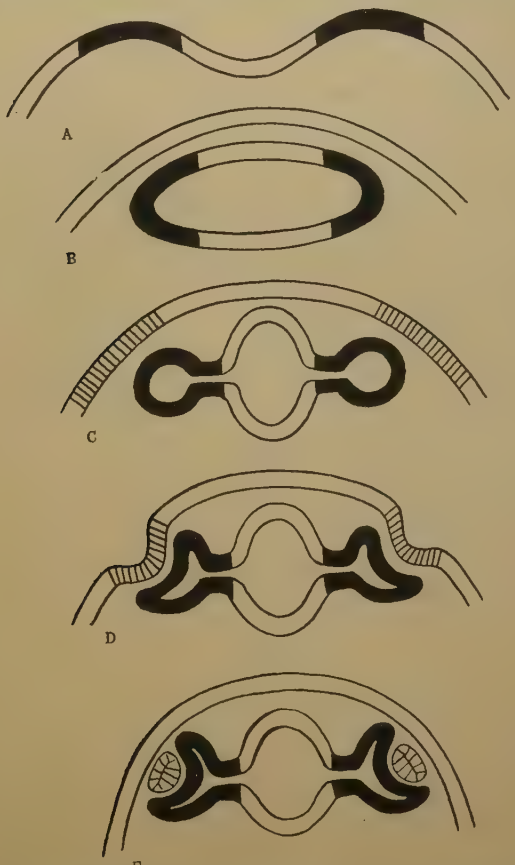

FIG. 134. — The development of the vertebrate eye. A, placodes before inclusion in neural tube. B, in neural tube. C, optic vesicles and lens placodes. D, optic cups and lens pits. E, optic cups and lens vesicles.

The lens. — The lens placode invaginates to form the **lens pit,** which then withdraws still further from the surface and becomes closed in by the union of its external lip to form the **lens vesicle.** The lens vesicle becomes solid by the elongation of the

cells on the internal side which assume a clear transparent appearance.

The optic cup. — The inner layer of the cup becomes the **sensory portion of the retina,** the outer layer the **pigmented portion.** It will be recalled that the sensory epithelium of the eye is inverted, and as a result the rods and cones, or sensory elements, of the retina are pointed away from the light. In the pigmented layer of the retina, melanin is secreted. Meantime the cavity of the optic cup becomes filled with a clear fluid secreted by the surrounding cells, which later becomes viscous and forms the **vitreous humor.**

The envelopes of the eyeball (Fig. 135). — Around the optic cup and stalk, a layer of mesenchyme accumulates, which later

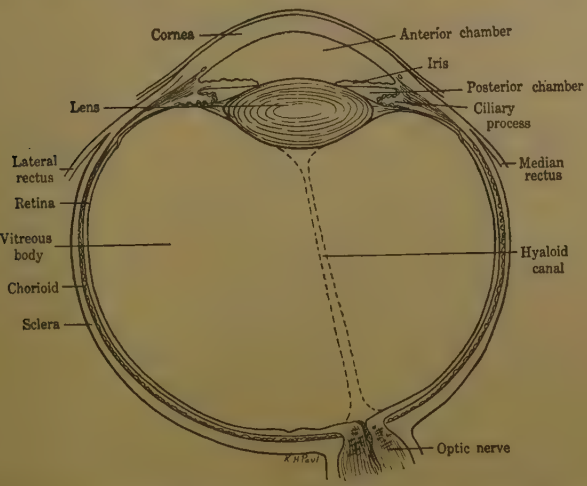

FIG. 135. — Diagram of human eye. Horizontal section. (After Howden, modified.)

differentiates into an inner delicate layer called the **chorioid** which contains pigment and capillaries and may be compared with the **pia mater** of the brain, and an outer dense layer known as the **sclera,** which may be compared with the **dura mater** of the brain. The external portion of the sclera over the lens makes contact with the epidermis and becomes transparent to form the **cornea.** The epidermis over the eye forms the **conjunctiva.** In some

vertebrates, sclerotic cartilage, or even bone, is formed, the vestige of an optic capsule. The edge of the chorioid, together with the marginal retina, gives rise to the **iris,** a circular curtain surrounding the opening of the cup which is called the **pupil** of the eye. The muscles of the iris are apparently of ectodermal origin. The iris divides the space between the lens and the cornea into two chambers, an **anterior** and a **posterior chamber,** which are filled with a fluid, the **aqueous humor.** The muscles of the eye are six in number, arising from the three head myotomes. They are innervated by the oculomotor, trochlear, and abducens nerves.

The optic nerve. — The afferent neurones pass from the retina into the optic cup and form a bundle which passes out through the chorioid fissure and into the optic stalk, and so to the optic chiasma on the floor of the diencephalon, where they cross and make their way to the optic lobes on the opposite side.

The lateral line system. — This is a diffuse sensory organ consisting of sense buds arranged in rows over the head and body of aquatic anamniota. Its function apparently is to detect slow vibrations in the water. The origin of the lateralis system is a lateral thickening of the sensory ectoderm which later breaks up into separate **suprabranchial placodes.** These are found in the embryos of the amniota but soon degenerate. The lateralis system is of particular interest inasmuch as the lateral thickening referred to is in some cases continuous with the otic placode which gives rise to the ear. The principal nerve supplying the lateralis system is the facial, although trigeminal, glossopharyngeal, and vagus often contains lateralis components.

The ear. — The ear, originally a part of the lateralis system, becomes differentiated into a **vestibular** equilibratory organ and the **cochlea** or organ of hearing. Three parts of the ear are distinguished (Fig. 137). The **inner ear,** giving rise to the vestibule and the cochlea, arises from an ectodermal **otic placode.** The **middle ear** first appears in the amphibia, and it is derived from the first visceral pouch (spiracle of fishes). The outer ear, found only in the amniotes, is a derivative of the first visceral groove and an outgrowth from the mandibular and hyoid arches.

The inner ear. — This originates from the otic placode, which invaginates to form an **otic pit** (Fig. 136) and later closes over to

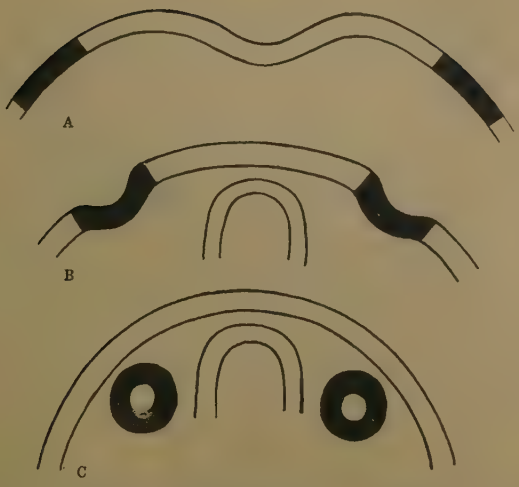

FIG. 136. — Development of the inner ear. A, otic placode. B, otic pit. C, otic vesicle.

FIG. 137. — Diagram of the human ear. Frontal section. (After Howden, modified.)

withdraw from the epidermis as the **otic vesicle.** In some vertebrates (elasmobranchs) the vesicle retains its connection with the exterior by means of a hollow stalk, the **endolymphatic duct.** Usually this connection is lost and the endolymph duct of the adult is a new formation. The vesicle divides into a ventral **saccule** and a dorsal **vestibule** or **utricle.** The saccule gives rise to the **endolymph** duct and the **lagena,** which in mammals becomes the coiled **cochlea** or organ of hearing, while the utricle gives rise by constriction to three **semicircular canals,** each with a dilation at one end, the **ampulla.** The sensory epithelium is restricted to the lagena and ampullæ. The cavity of these structures is known as the **membranous labyrinth,** and contains a fluid, the **endolymph.** Concretions, the **otoliths,** may appear in the endolymph of the vestibular portion. Around this labyrinth the otic capsule, or **skeletal labyrinth,** is formed. This later ossifies to give rise to the otic bones. The skeletal labyrinth contains a fluid known as the **perilymph.** In vertebrates with a middle ear, two openings are formed in the skeletal labyrinth, the **fenestra rotunda,** closed by a membrane, and the **fenestra ovale,** into which the stapes projects. The acoustic nerve, which is ganglionated, divides into a vestibular and a cochlear nerve, each with its separate ganglion.

The middle ear. — The middle ear arises from the first visceral pouch, which constricts into a proximal **auditory tube** and a distal **tympanic cavity** which is separated from the exterior by the **tympanic membrane,** a persistent closing plate formed from ectoderm and endoderm. Through the tympanic cavity there is a chain of bones connecting the tympanum with the fenestra ovalis. In anura and sauropsids, this chain of **auditory ossicles** consists of the columella and stapes (hyomandibular). In the mammals, the columella is replaced by the incus and malleus, equivalent to two other jaw bones, the quadrate and articulare, respectively. The muscles of the middle ear, **tensor tympani** and **stapedial muscles,** arise from the mesoderm of the mandibular and hyoid arches, respectively, and are innervated by the facial and glossopharyngeal nerves.

The outer ear. — The external ear consists of the **external auditory meatus,** derived from the first visceral groove, and the **pinna,** which arises from tubercles on the mandibular and hyoid

arches. It is composed of mesoderm and ectoderm, contains muscles, and is strengthened by cartilage. The innervation is from the facial nerve.

The frog (*See also Chapter VIII*). — In the development of the nose, the nasal groove stage is suppressed. Instead, a thickening develops from the olfactory pit into the mouth as far as the pharynx. This acquires a lumen which connects the olfactory pit to the pharynx. The development of the eye presents no especial peculiarities. The endolymph duct is a dorsal evagination from the otocyst. The semicircular canals are each formed by the appearance of a pair of ridges in the cavity of the utricle which fuse to enclose the cavity of the canal. The saccule gives rise to two ventral diverticula, the cochlea and basilar chamber. The function of the latter is unknown. The tubo-tympanic cavity arises from the first visceral pouch, which in the frog is vestigial and has no cavity. From this rudiment a strand of cells grows dorsad and later acquires a lumen. It loses its connection with the pharynx and moves backward to the ear region where it acquires a secondary connection with the pharynx. The tympanic membrane is apparently entirely ectodermal. The columella, which connects the tympanum with the inner ear, arises from two primordia: the inner stapedial plate, which is a part of the otic capsule; and a cartilage derived from the palatoquadrate bar which corresponds to the hyomandibular of fishes. The lateral line organs arise from the fragmentation of a placode known as the placode of the tenth cranial nerve, which innervates this series. Similar epibranchial placodes appear on the head and are innervated by the seventh and ninth nerves. They are larval sense organs and disappear at metamorphosis.

The chick (*See also Chapter IX*). — The chick has a cleft palate due to the incomplete fusion of the palatine processes of the maxillæ. Jacobsen's organ makes a short appearance as a vestigial organ but disappears before hatching. The eye possesses three eyelids, the third (**nictitating membrane**) arising from a separate fold inside that which forms the upper and lower lids. The **pecten** is an enigmatic vascular plate in the vitreous humor, from mesenchyme which enters the chorioid fissure. The endolymph duct arises from the dorsal wall of the otocyst. The semicircular canals arise as outpocketings of the otocyst

prior to its separation into utricle and saccule. The cochlea is more highly developed than in the frog. The tubo-tympanic cavity arises from the first pharyngeal pouch. The tympanum is formed from ectoderm and endoderm and includes a middle layer of mesenchyme. The columella arises from a stapedial plate and hyomandibular cartilage. The external auditory meatus is short, and no pinna is developed.

Man (*See also Chapter X*). — The organ of Jacobsen is rudimentary and may completely disappear in the adult. A **plica semilunaris** is the representative of the nictitating membrane. The cochlea is highly differentiated. The tube and tympanic cavity form from the first visceral pouch. The tympanum apparently is composed of all three germ layers. The stapes is derived from the second visceral arch, while the malleus and incus arise from the first and represent the quadrate and articular, respectively. The pinna arises from elevations on the mandibular and hyoid arches.

Summary. — The ectoderm gives rise to the epithelial linings of the following structures:

A. The epidermis, with the
- **apertures of**
  - **Oral cavity**
  - **Visceral clefts**
  - **Cloaca**

B. The neural plate

1. Neural tube
- **Brain and cranial nerves**
  - **Prosencephalon**
    - **Telencephalon**
    - **Diencephalon**
  - **Mesencephalon**
  - **Rhombencephalon**
    - **Metencephalon**
    - **Myelencephalon**
- **Cord and spinal nerves**

2. Neural crest
- **Ganglia**
  - **Cerebrospinal**
  - **Autonomic**

C. The sense plate
   1. Nose
   2. Eye (chorioid and sclera from mesoderm)
   3. Ear (middle ear from endoderm, ossicles from mesoderm).

REFERENCES

Brachet, A. 1921. Traité d'Embryologie des Vertébrés, Part II, Bk. 1, Chap. 4.
Graham-Kerr, J. 1919. Textbook of Embryology, Chap. 2.
Hertwig, O. 1906. Handbuch, Book II, Chaps. 5–10.
Jenkinson, J. W. 1913. Vertebrate Embryology, Chap 7.
Keibel and Mall. 1910–1912. Human Embryology, Chaps. 14 and 16.
Kellicott, W. E. 1913. Chordate Development.
Kingsley, J. S. 1926. Comparative Anatomy of Vertebrates.
Lillie, F. R. 1919. The Development of the Chick.
McMurrich, J. P. 1923. The Development of the Human Body.
Strong, O. S. 1921. The Nervous System, being Chap. 17 of Bailey and Miller, Textbook of Embryology, 4th Ed.

PART III

ATLAS OF SECTIONS

CHAPTER VIII

THE ANATOMY OF FROG EMBRYOS

In earlier chapters we have discussed the fertilization of the frog's egg (page 37), its cleavage (pages 63, 70), and germ-layer formation (pages 77, 82), and have observed that while the germ layers are being laid down the process is complicated by the early localization of some of the organ systems, notably the sensory-nervous complex (pages 86, 88, 91). In this account of later organogeny, three stages of development seem especially significant: first, an early embryo of about 3 mm. body length in which the visceral grooves are apparent, a stage attained in *Rana pipiens* about the second day after the eggs are laid; second, the newly hatched larva of about 6 mm. with external gills developing, about two weeks old; third, a young " tadpole " stage of about 9 mm. with the opercula covering the internal gills, about the age of one month.

These stages are easily identified even though the lengths and ages can be given only approximately, for the rate of development is greatly influenced by the prevailing temperature, while the size of the tadpole is determined largely by external factors, such as the amount of food available.

A. THE EARLY EMBRYO (3 MM.)

External form. — This stage corresponds approximately to the embryo of 3½ mm. described by Marshall. The head region, through its more rapid growth, has become easily distinguishable from the trunk, which bulges ventrally on account of the large amount of contained yolk, and a well-marked tail bud is present. The neural folds have fused throughout their length, and enclosed the blastopore. In the head the stomodæum appears

as an antero-posterior slit on the anterior ventral surface, and is enclosed by ridges identifiable as the maxillary processes and mandibular arches. On either side and slightly ventral to the stomodæum, are the primordia of the oral gland, while at the dorso-lateral margins the olfactory placodes have begun to evaginate. Lateral bulges on either side of the head are due to the developing optic vesicles. The gill plate shows five visceral grooves posterior to the mandibular arch. Immediately behind the last arch, a swelling is caused by the developing pronephros.

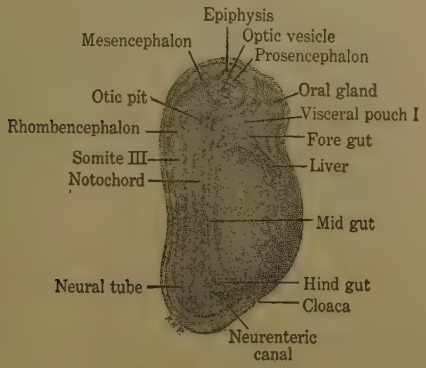

FIG. 138. — Lateral view of 3 mm. frog embryo. Cleared specimen. ×15.

Dorsally, slight furrows indicate the boundaries of thirteen somites. Beneath the tail bud, the proctodæum has united with the hind-gut to form the cloacal aperture.

Endodermal derivatives. — The anterior portion of the gastrocœle is now a large fore-gut with a thin-walled lining. From this, on either side, the beginnings of three visceral pouches can be seen. From the fore-gut a narrow evagination grows backward into the floor of the mid-gut as the primordium of the liver. The mid-gut is distinguishable by its relatively narrow lumen and thick yolk-laden floor. The small but thin-walled hind-gut opens above into the neurenteric canal by which it is connected with the neurocœle, and opens ventrally to the exterior by way of the proctodæum. An enigmatic rod, the **hypochord,** is found beneath the notochord. It is apparently of endodermal origin and disappears soon after hatching.

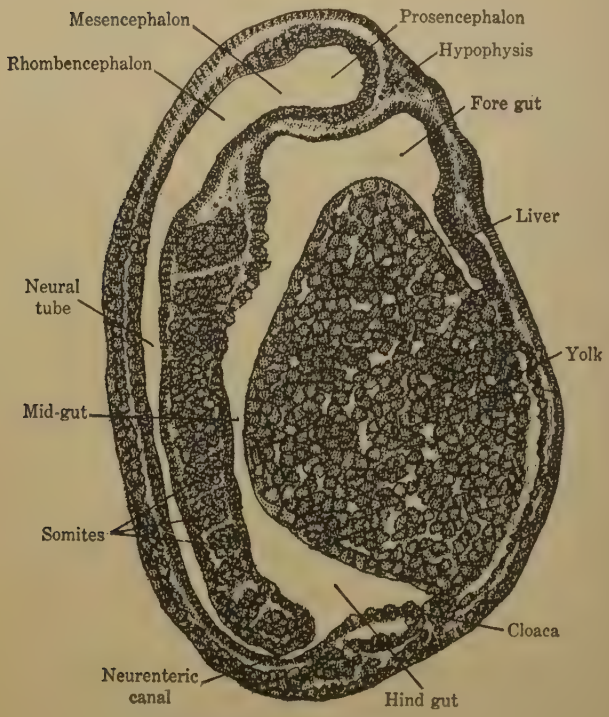

FIG. 139. — Sagittal section of 3 mm. frog embryo. ×50.

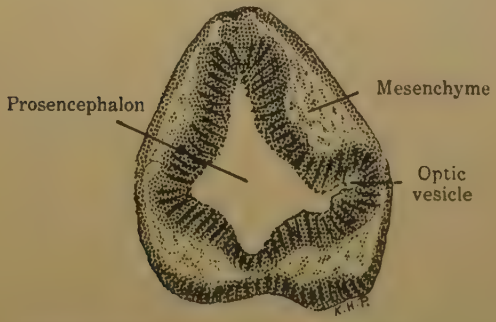

FIG. 140. — Transverse section through optic vesicle of 3 mm. frog embryo. ×50.

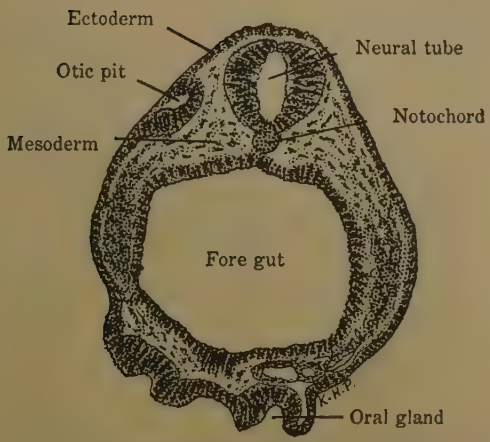

FIG. 141. — Transverse section through otic pit of 3 mm. frog embryo. ×50.

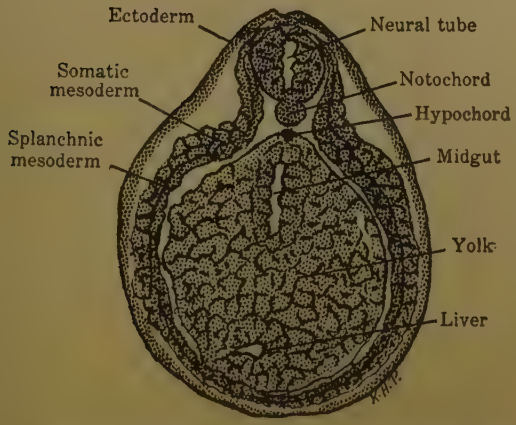

FIG. 142. — Transverse section through mid-gut of 3 mm. frog embryo. ×50.

Mesodermal derivatives. — The notochord is large and vacuolated and enclosed by two sheaths. The somites have now attained their maximum number (13) in the trunk, but are not yet distinguishable in the tail region. The mesomere, after a temporary division into nephrotomes, is now reunited into a

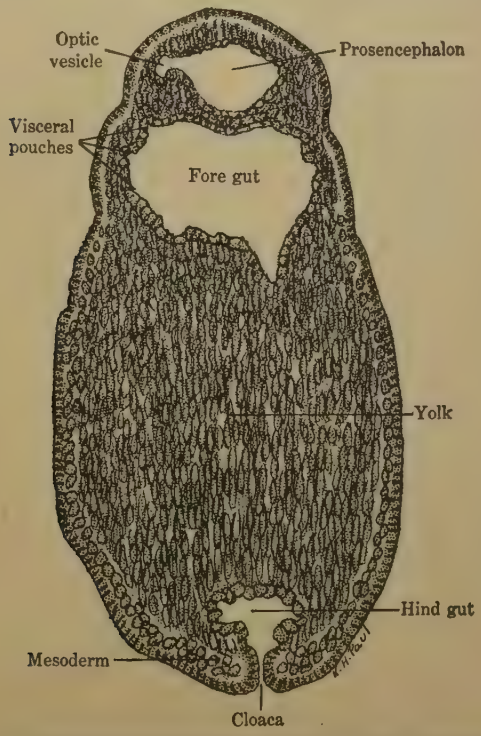

FIG. 143. — Frontal section through optic vesicle of 3 mm. frog embryo. ×50.

nephrotomal band in which spaces have appeared opposite the second, third, and fourth somites, indicative of the pronephric tubules which are to develop. A thickening along the nephrotomal band immediately below the ventro-lateral margins of the somites is the primordium of the pronephric duct. Immediately below the floor of the fore-gut, the hypomere has separated into dorsal splanchnic and ventral somatic layers, while the contained

space is the beginning of the pericardial cavity, the only region of the cœlom yet apparent.

Ectodermal derivatives. — The epidermis at this stage is ciliated. The neurocœle, as has been remarked above, is connected with the hind-gut by the neurenteric canal. At the anterior end, the brain is distinguishable by its relatively larger lumen and by the cranial flexure over the anterior end of the notochord. The divisions between the three primary vesicles are not marked by the constrictions characteristic of many vertebrates, but are distinguished by the following points of reference: the prosencephalon extends to a line projected from a thickening on the floor known as the **tuberculum posterius** to a point just in front of a similar thickening on the dorsal wall; the mesencephalon, from the boundary of the prosencephalon to a line connecting the tuberculum and a point just behind the dorsal thickening; the rhombencephalon merges imperceptibly into the spinal cord. From the prosencephalon, the optic vesicles extend on either side and cause the external bulges already noted. From the ventral side of the prosencephalon, a depression, the infundibulum, extends towards the hypophysis, which in the frog grows inward as a solid wedge of ectodermal cells anterior to the stomodæum. Dorsally, the epiphysis appears as a median evagination. The ear is in the otic pit stage.

B. THE LARVA AT HATCHING (6 MM.)

External form. — Although the larva, if it may be so called, has emerged from the protecting membranes of egg jelly, the mouth has not yet opened and for several days the yolk is still the sole source of food. The head region is still easily distinguishable from the trunk, while the tail has increased greatly in length and has become bilaterally compressed. In the head, the stomodæal pit has deepened at the anterior end, and the maxillary processes and mandibular arches are more sharply sculptured. The invagination of the olfactory placodes has continued to the point where they may be called pits, connected to the anterior margins of the stomodæal pit by oro-nasal grooves. The bulge of the eye is still prominent. The primordia of the oral glands have fused to form a U-shaped sucker ventral and posterior to the stomodæum. The visceral grooves are still sepa-

rated from the visceral pouches by closing membranes, while on the third and fourth arches external gills have appeared. Behind them the pronephric elevation is well marked, and continues backward as a slight ridge marking the pronephric duct. Intersomitic grooves are still apparent. On the ventral side at the base of the tail is the cloacal aperture.

Endodermal derivatives. — On either side of the fore-gut are to be seen five visceral pouches, although they would hardly be recognized as such since they are so compressed. A groove on

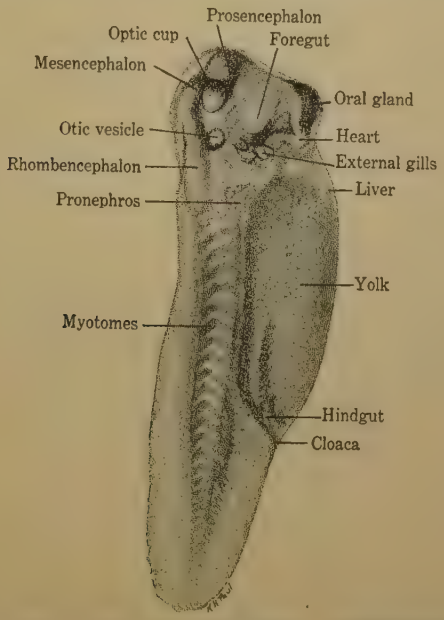

Fig. 144. — Lateral view of 6 mm. frog embryo. Cleared specimen. ×15.

the ventral side of the pharyngeal cavity is the primordium of the thyreoid gland. At this stage also, the dorsal epithelial bodies of the first two visceral pouches (hyomandibular and first branchial) may be distinguished. The liver diverticulum has increased in length. The hind-gut has lost its connection with the neurocœle through the occlusion of the neurenteric canal, but now receives the posterior ends of the pronephric ducts.

Mesodermal derivatives. — The notochord has grown back into the tail. The somites have now become differentiated into the myotomes, dermatomes, and sclerotomes, while from the myotomes muscle cells have been formed. The pronephros is now established. There are three pronephric tubules, each opening into the cœlom by means of a ciliated nephrostome. Opposite these, a mass of capillaries, connected with the dorsal aorta, forms the so-called glomus, equivalent to the separate glomeruli of other vertebrates. The pronephric tubules grow backward into the pronephric ducts, which have acquired lumina. At the time of hatching, the primordia of the heart have fused to form a tube, twisted slightly and almost S-shaped, suspended in the pericardial cavity by a dorsal mesocardium. Two regions may be distinguished, the posterior atrium and anterior ventricle. From the ventricle leads the bulbus, arising from the fusion of paired primordia. This connects with the dorsal aorta, also the result of fusion, by means of aortic arches in the third and fourth visceral arches (vestiges of the first and second aortic arches have already appeared and disappeared). At a slightly later stage, loops from these arches will grow out into the external gills to form a branchial circulation. The anterior ends of the dorsal aortæ are prolonged to form the internal carotids, while the posterior ends unite directly above the heart, and just after uniting give off the glomi on either side. Both the somatic and splanchnic venous systems are represented at this stage. Two vitelline veins unite to enter the heart at the sinus venosus. The cardinal veins at this time are represented by irregular lacunar spaces in the head and near the pronephros.

Ectodermal derivatives. — The epidermis is still ciliated. From the prosencephalon the thin-walled cerebral vesicle has appeared. The epiphysis is well marked, and the infundibulum is in contact with the hypophysis. At this time the primordia of cerebrospinal nerves may be distinguished. In the spinal nerves, dorsal roots arise from the ganglia produced by the segmentation of the neural crest while the ventral roots arise from neuroblasts in the spinal cord. In the head, four ganglia arise and with each is associated a placode of nervous ectoderm. From the first ganglion and placode, the trigeminal (V) nerve arises. The second combination gives rise to the facial (VII) and acoustic

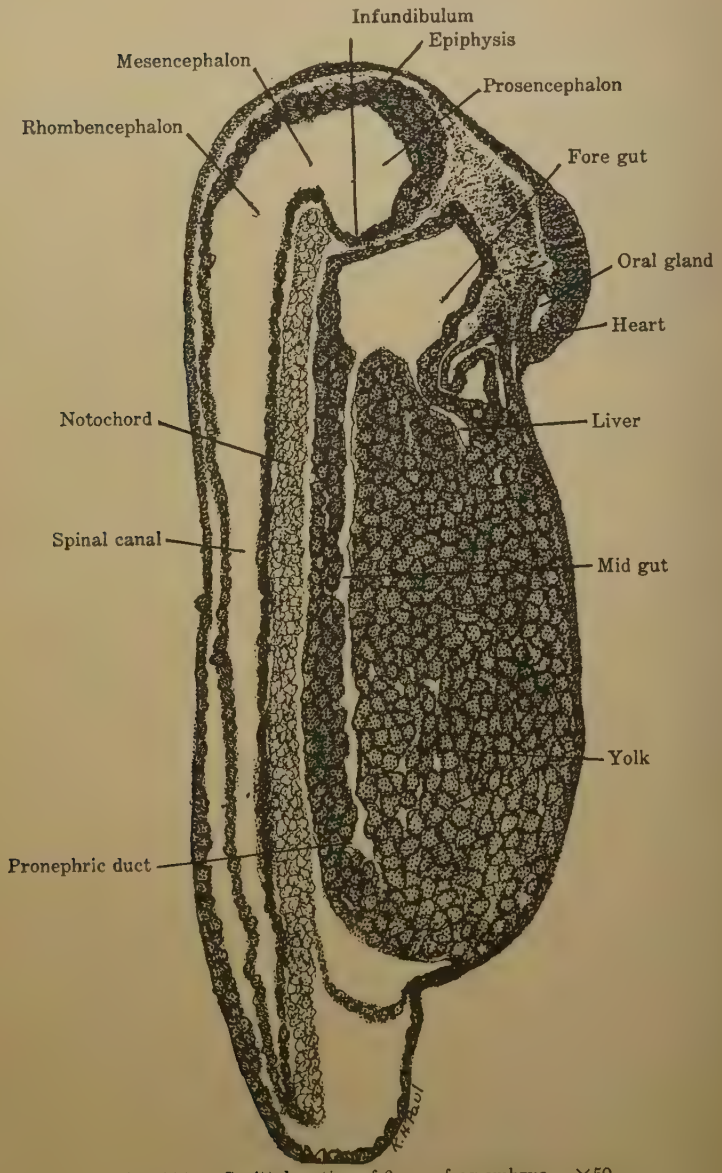

FIG. 145. — Sagittal section of 6 mm. frog embryo. ×50.

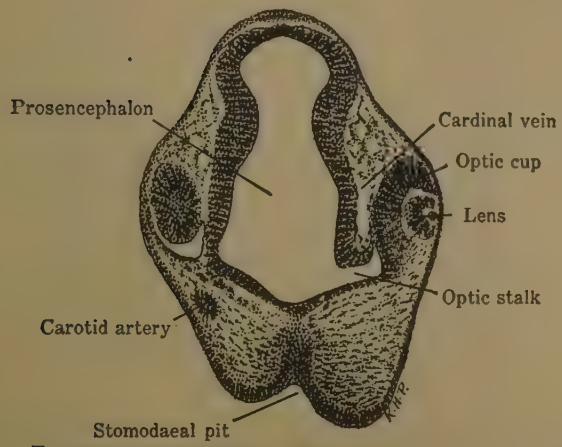

FIG. 146. — Transverse section through optic cup of 6 mm. frog embryo. ×50.

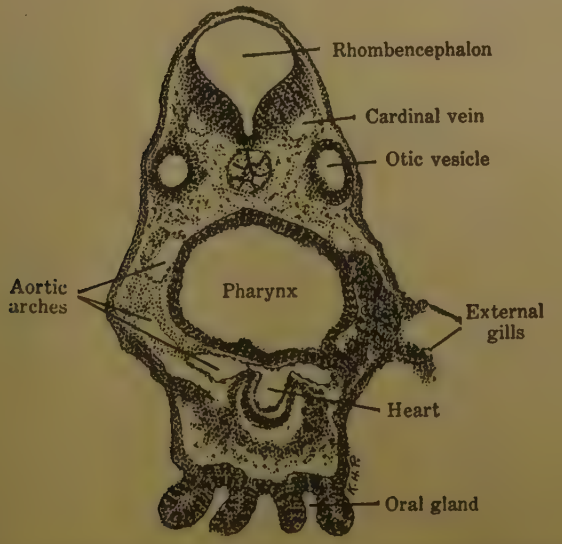

FIG. 147. — Transverse section through otic vesicle of 6 mm. frog embryo. ×50.

(VIII) cranial nerves, while the remainder of this placode invaginates to form the otic vesicle. The third ganglion and placode produce the glossopharyngeal (IX) cranial nerve, and the fourth gives rise to the vagus (X). The fourth placode grows back as far as the tail, giving off as it goes small groups of cells which later become the lateral line organs of the trunk. Those of the head arise from the second and third placodes. At this

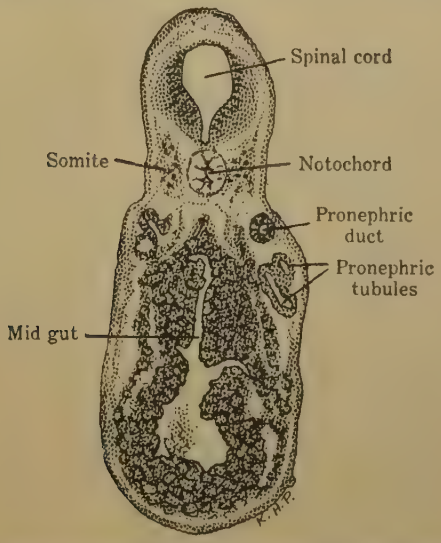

FIG. 148. — Transverse section through pronephros of 6 mm. frog embryo. ×50.

time also, ganglion cells are migrating toward the dorsal aorta to aggregate as the ganglia of the autonomic nervous system. The eye is well advanced in development, as the optic vesicles have invaginated to form the optic cup and the lens placode has separated from the epidermis and acquired a cavity. The ear is in the otic vesicle stage with an endolymphatic duct. The nose is still represented by the nasal pits. From the prolongation of the fourth placode referred to above, the lateral line system is in process of formation.

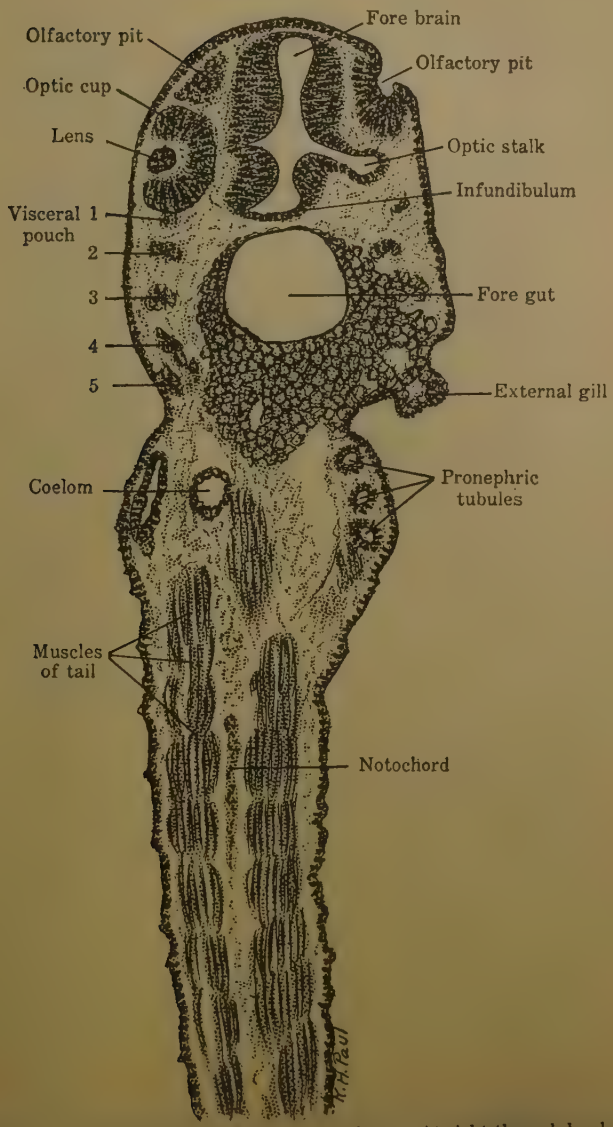

FIG. 149. — Frontal section of 6 mm. frog embryo. At right through level of olfactory pit. At left through level of optic cup. ×50.

C. THE YOUNG TADPOLE (9 MM.)

External form. — The head and trunk are now fused into a common ovoid mass, sharply distinguished from the long bilaterally compressed tail. The mouth is open and equipped with horny raspers, while the oral gland is reduced to two vestiges on the ventral side of the head. On the dorsal surface, the large eyes, now functional, protrude slightly. Anterior to these are the external openings of the nasal tubes (external nares). The external gills, which were developing at hatching, have now degenerated and been replaced by internal gills concealed from view by the opercula. On the left side, the opercular aperture serves as a means of egress for the water from which the gills obtain their oxygen. The tail, now two-thirds the length of the tadpole, has a dorsal and a ventral fin. Close to the margin of the latter, at the base of the tail, is the cloacal opening.

Endodermal derivatives. — The mouth has been formed by the breaking through of the oral membrane. From the pharynx, all the visceral pouches except the hyomandibular (I, II, III, IV branchials) open to the exterior as visceral clefts; and demibranchs have arisen on the anterior and posterior margins of the third, fourth, and fifth visceral arches and on the anterior margin of the sixth. These are the internal gills which hang down into the opercular cavity. The epithelial bodies from the hyomandibular pouch have disappeared. Those from the second pouch form the thymus gland, while similar buds arise from the third and fourth but presently disappear. The ventral epithelial bodies of the second pouch are said to give rise to the carotid gland, and those of the third and fourth to "parathyreoids." The fifth pouch never gains communication with the exterior but gives rise to the ultimobranchial bodies. The thyreoid is now separated from the pharynx. In the tadpole the pulmonary organs consist of a pair of thin-walled sacs, the lungs, arising from a laryngeal cavity connected with the pharynx by a narrow opening, the glottis. Posterior to the pharynx comes the œsophagus, which was occluded just before the opening of the mouth but now possesses a narrow lumen opening into the stomach, which is not greatly dilated. The diverticulum, which formerly represented the liver, persists as the gall-bladder and

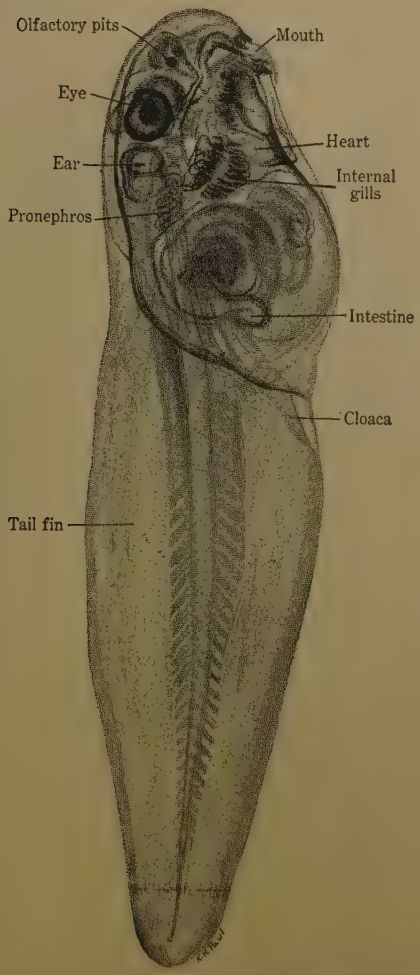

FIG. 150. — Lateral view of 9 mm. frog larva. Cleared specimen loaned by Dr. A. R. Cahn. ×15.

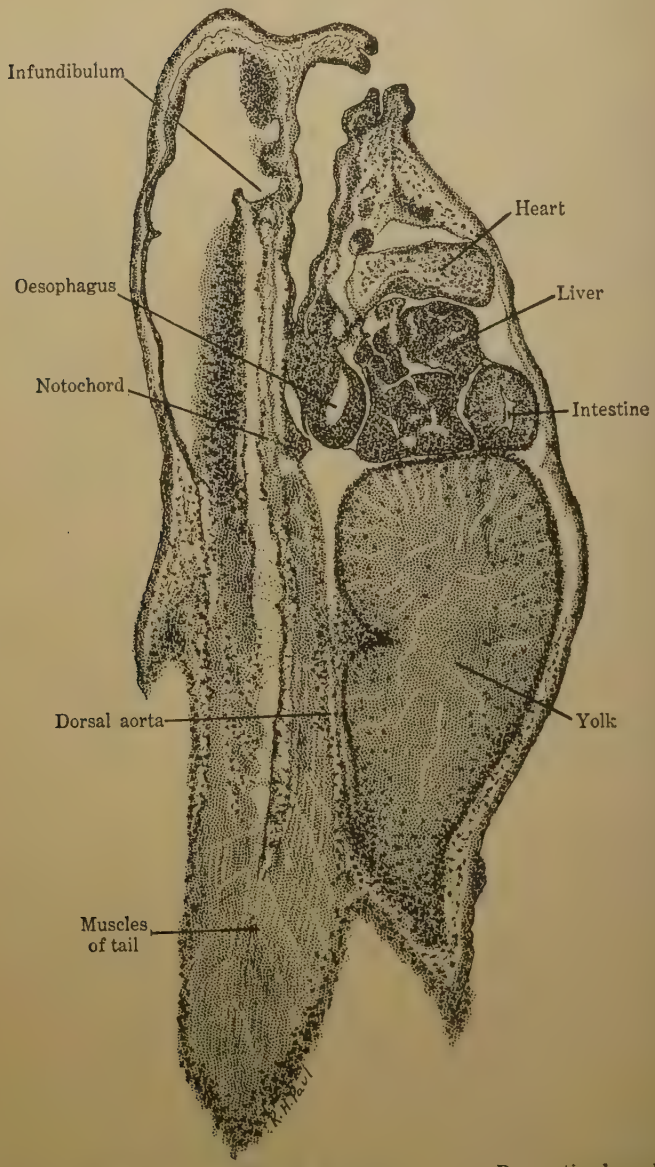

FIG. 151. — Sagittal section of 9 mm. frog larva. ×40. Preparation loaned by Dr. A. R. Cahn.

ductus choledochus, relatively small in comparison with the great glandular mass of the liver. Although the pancreas arose from paired primordia of the duodenum, these have now shifted their position so that their ducts open into the ductus choledochus. The intestine is extremely long and coiled into a double spiral. It terminates in a slightly dilated rectum, opening into the cloacal cavity which also receives the pronephric ducts and opens to the exterior by the cloacal aperture.

Mesodermal derivatives. — The notochord has elongated toward the posterior end, accompanying the growth of the tail. The two most anterior somites have disappeared, leaving eleven

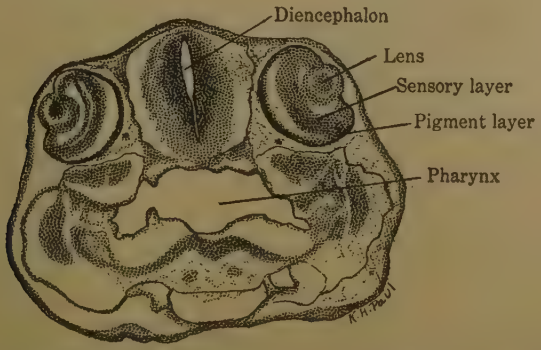

FIG. 152. — Transverse section through eye of 9 mm. frog larva. ×40. Preparation loaned by Dr. A. R. Cahn.

in the trunk region and a much larger and variable number in the tail. In the latter organ the myotomes have given rise to the epaxial and hypaxial musculature. The pronephros has become larger and more complicated through the branching of the pronephric tubules. The cœlom consists of a pericardial cavity containing the heart, whose myocardia have disappeared, and an abdominal cavity in which the gut is suspended by the dorsal mesentery. These cavities are still continuous up to the time of metamorphosis. In the heart the sinus venosus is now a large transverse sac; the atrium is partially divided by the interatrial septum; the ventricle has thick muscular walls; and the short bulbus opens into the ventral aorta which is divided into proximal and distal portions by a pair of valves.

The ventral aorta is divided into four afferent branchial arteries, the ventral portions of aortic arches III–VI. From these the blood passes through the internal gills by means of capillaries and is conveyed to four efferent branchial arteries, the dorsal portions of the aortic arches referred to above, which in turn lead to the dorsal aortæ. The carotid arteries are connected in front of and behind the infundibulum by **commissural vessels,** and continue forward as the **anterior cerebral arteries.** From the anterior commissure the **basilars** run backward and the **anterior**

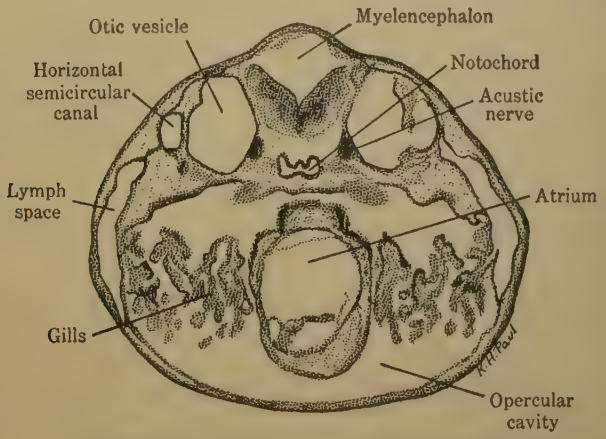

Fig. 153. — Transverse section through otic vesicle of 9 mm. frog larva. ×40. Preparation loaned by Dr. A. R. Cahn.

palatines forward. The **pharyngeal artery,** running forward from the dorsal aorta, at a point just posterior to the anterior commissure, represents the dorsal portion of the mandibular arch; the **lingual artery** arises independently and unites with the first efferent branchial. From the efferent branchial arteries of the sixth arch, the pulmonary arteries grow backward to the lungs. The vitelline veins have been broken up, by their inclusion in the developing liver, into the hepatic veins, opening into the sinus venosus, and the portal veins from the intestine. The anterior cardinal veins are formed by the union of the superior jugular and facial veins and empty into the common cardinals. From the ventral side of the head the inferior jugulars drain into

the common cardinals. The posterior somatic veins are the posterior cardinals, which return the blood from the region of the pronephros into the common cardinals. The lymphatic vessels of the tadpole have arisen from the confluence of numerous, small intercellular lacunæ of the mesenchyme.

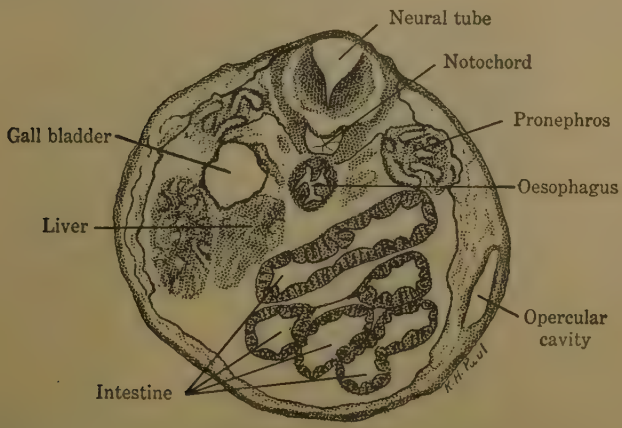

FIG. 154. — Transverse section through pronephros of 9 mm. frog larva. ×40. Preparation loaned by Dr. A. R. Cahn.

The ectodermal derivatives. — The epidermis is no longer ciliated. The cerebral vesicle is now sub-divided into right and left portions, while immediately behind this is the chorioid plexus of the diencephalon. The pineal gland is connected with the diencephalon by a small stalk; the pituitary gland has lost all connection with the exterior. In the mesencephalon the optic lobes are just apparent. The metencephalon is distinguishable by the thickness of its walls as compared with the chorioid plexus of the myelencephalon. All cranial nerves and spinal nerves are now established. The eye now contains all elements necessary for functioning; rods and cones of the sensory layer connect with the neurones of the optic nerve; pigment is deposited in the pigment layer; the chorioid and sclerotic layers have been formed from mesenchyme; the lens is transparent, as is the cornea formed from the ectoderm. The otocyst is partially divided by a dorsal partition into an outer saccule and inner

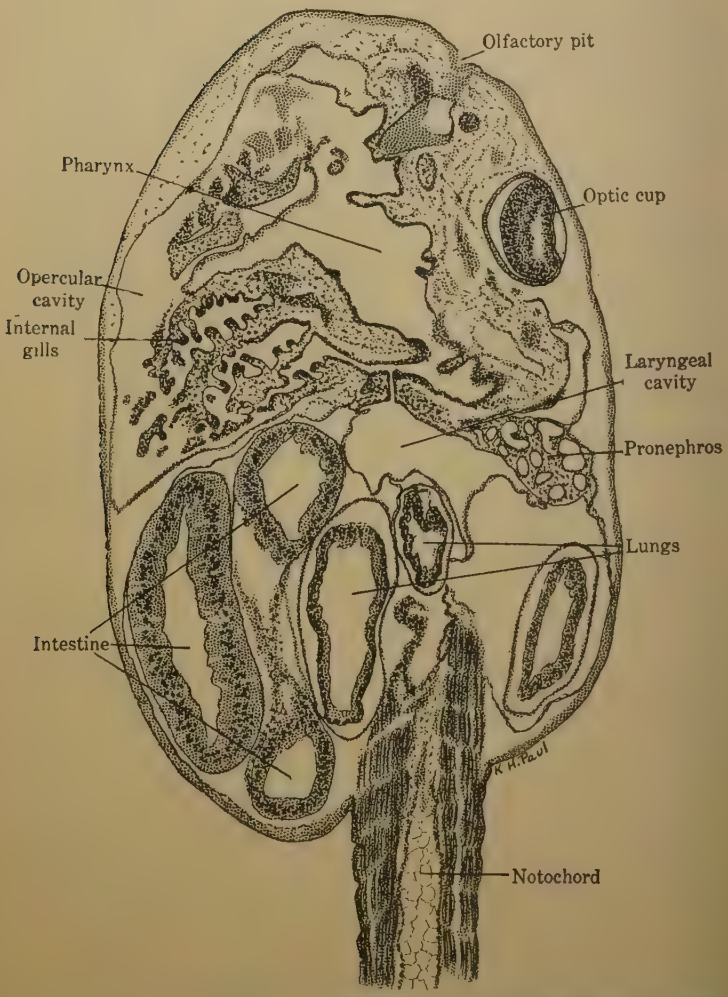

Fig. 155. — Frontal section of 9 mm. frog larva. At right through level of olfactory pit and eye. At left through level of internal gills. ×40. Preparation loaned by Dr. A. R. Cahn.

utricle. The nasal pits have grown backward as solid rods which by now have acquired lumina and openings into the pharynx (the internal nares).

REFERENCES

Jenkinson, J. W. 1913. Vertebrate Embryology, Chap. 7.
Marshall, A. M. 1893. Vertebrate Embryology, Chap. 3.
Morgan, T. H. 1897. The Development of the Frog's Egg.
Zeigler, H. E. 1902. Lehrbuch der vergleichenden Entwickelungsgeschichte der niederen Wirbeltiere.

CHAPTER IX

THE ANATOMY OF CHICK EMBRYOS

The traditional stages in the development of the chick (*Gallus domesticus*) for laboratory practice are those at the end of each of the first three days of incubation. So many important changes take place during the second day, however, that it is advisable to study an additional stage intermediate between twenty-four and forty-eight hours in age. The chick of thirty-three hours is selected because the form of the embryo is not yet affected by torsion or flexure, and the head fold of the amnion has not yet slipped over the head of the chick.

As it is a well-known fact that in these first few days of incubation, embryos of the same age have attained varying degrees of development, the length of the embryo has been proposed as a mark of identification. The flexures of the body, however, make this standard impracticable, and the remaining alternative is to select the specific development of some particular structure as a basis of arrangement. For this purpose the number of somites, suggested by Lillie, is admirable. Still, it must be remembered that on account of the effect of temperature upon the rate of development, the number of somites is not correlated exactly with the number of hours of incubation, as may be seen from the following table.

TABLE IX

	Duval	Keibel	Lillie	Patten
About 24 hours	Fig. 76 (24 hrs. 6 S)	Fig. 9, 9A (24 hrs. 7–8 S)	Fig. 59 (25 hrs. 7 S)	Fig. 36 (27 hrs. 8 S)
About 33 hours	Fig. 93 (33 hrs. 16 S)	Fig. 10, 10A (32 hrs. 9 S)	Fig. 63 (33 hrs. 12 S)	Fig. 39 (33 hrs. 12 S)
About 48 hours	Fig. 109 (48 hrs. 27–28 S)	Fig. 16, 16A (52 hrs. 27 S)	Fig. 93 (48 hrs. 27 S)	Fig. 55 (55 hrs. 29 S)
About 72 hours	Fig. 115 (68 hrs. 47 S)	Fig. 18, 18A (67 hrs. 35–37 S)	Fig. 117 (72 hrs. 35 S)	Fig. 63 (72 hrs. 36 S)

A. THE TWENTY-FOUR HOUR STAGE

At the end of the first day of incubation, the chick embryo has completed the period of cleavage (pages 65, 70) and germ-layer formation (pages 78, 85), and is in the early stages of organogeny.

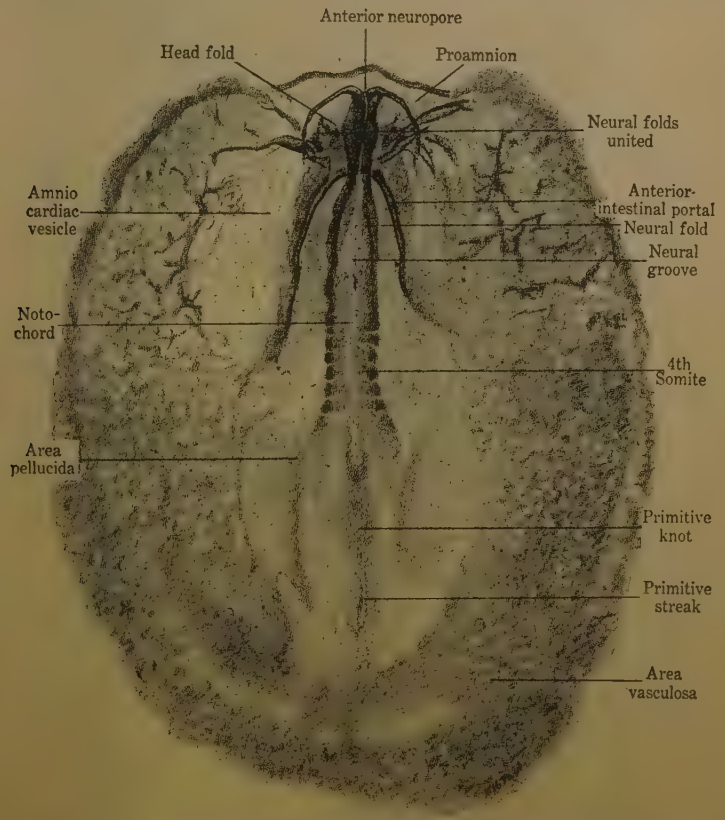

FIG. 156. — Dorsal view of 24 hour chick embryo. Cleared preparation. ×50.

External form. — The embryo, 3.3 mm. in length, lies along the axial line of the slipper-shaped area pellucida which in turn is surrounded by the crescent-shaped area vasculosa, whose anterior horns, separated by the proamnion, reach about to the level of

tip of the head. At the anterior end, the head fold of the embryo is lifted above the proamnion from which it is separated by the subcephalic pocket. In the head fold is contained the fore-gut, .59 mm. in length, which opens at its posterior end into the yolk

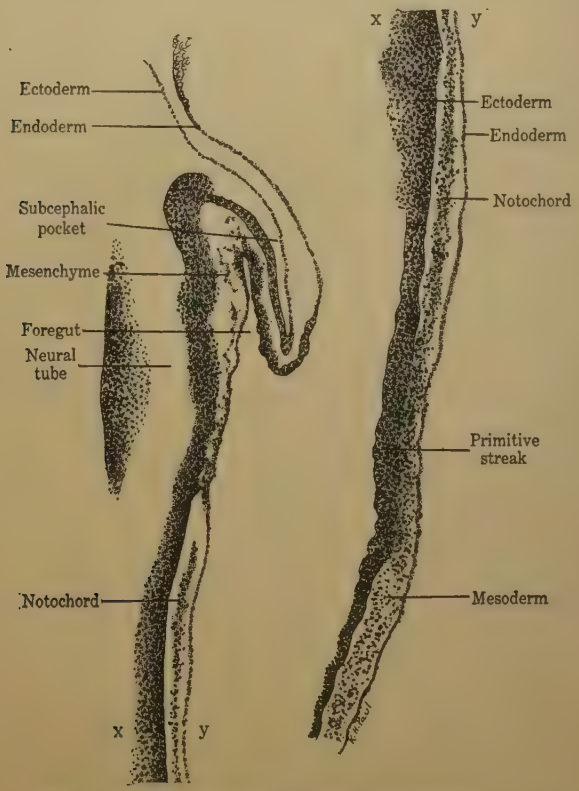

FIG. 157. — Sagittal section of 24 hour chick embryo. ×75.

cavity by means of the anterior intestinal portal. On either margin of the portal the primordia of the vitelline veins are to be recognized in thick bands of splanchnic mesoderm. The neural plate has already given rise to the neural folds which extend back as far as the first somite. They have united just posterior to the region where the optic vesicles are to appear and

thus have given rise to a neural tube 0.3 mm. in length, which is widely open in front and behind as the anterior and posterior neuropores, respectively. Behind the neural folds the axial mesoderm (epimere) is segmented into six somites. Between the neural folds the notochord can be recognized as a faint line which joins, at its posterior end, the primitive streak, now reduced to .83 mm. in length.

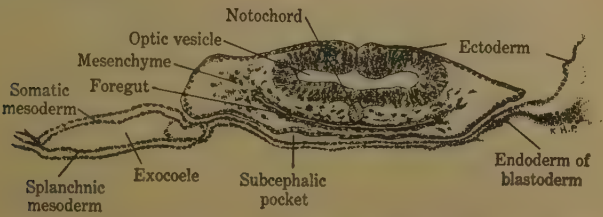

FIG. 158. — Transverse section through optic vesicles of 24 hour chick embryo. ×75.

Endodermal derivatives. — The only differentiation which has taken place in the endoderm consists of the establishment of the fore-gut by means of the folding off of the head from the proamnion. As this process continues the fore-gut will be lengthened at the expense of the widely open mid-gut, and the anterior intestinal portal will progress steadily backward.

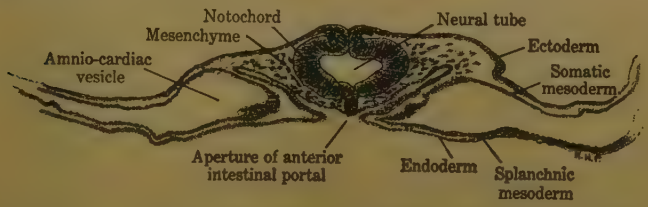

FIG. 159. — Transverse section through intestinal portal of 24 hour chick embryo. ×75.

Mesodermal derivatives. — The mesoderm does not extend into the head proper, but a loose aggregate of mesenchyme derived from the mesoderm is present. Posterior to the head the axial mesoderm is divided into six somites. Transverse sections show that each has a minute cavity, or myocœle. The intermediate mesoderm (mesomere) does not divide into nephro-

tomes as in the frog. The lateral hypomere is divided into the somatic and splanchnic layers. In the latter, numerous blood islands appear and give the characteristic mottled appearance to the area vasculosa. The cœlom of the embryo is continuous

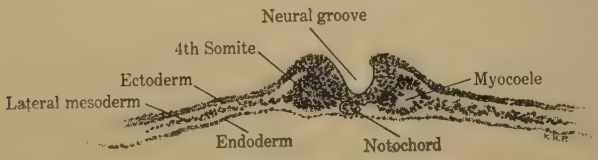

Fig. 160. — Transverse section through fourth somite of 24 hour chick embryo. ×75.

with that of the extra-embryonic regions, or exocœle. In the region on either side of the head, between the proamnion and the intestinal portal, the cœlom is distended into an amniocardiac vesicle, so-called because the somatopleure will contribute to the head fold of the amnion, while the splanchnic mesoderm will give rise to the primordia of the heart, and the cavities of the vesicles will become confluent to form the pericardial cavity. The notochord, from its point of origin, the primitive streak, extends forward into the head.

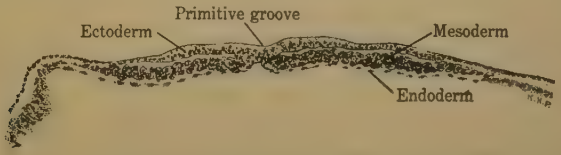

Fig. 161. — Transverse section through primitive streak of 24 hour chick embryo. ×75.

Ectodermal derivatives. — The ectoderm at this stage consists of the elongate neural plate, with its groove and folds which are already in process of fusion, and the epidermis or non-nervous ectoderm.

B. THE THIRTY-THREE HOUR STAGE

External form. — In the chick embryo, after thirty-three hours' incubation, the length has increased to 4.3 mm. There is a slight bending of the head downward over the end of the notochord, foreshadowing the cranial flexure. The area vasculosa,

in which the blood islands are being converted into capillaries, now has grown in toward the embryo, so that the area pellucida persists only around the head and tail regions. The anterior horns of the area vasculosa have met in front, completely inclosing the proamnion. The head has increased in length not only by actual forward growth but by the backward extension of the lateral margins of the head fold, so that the enclosed fore-gut is now 1 mm. long. The vitelline veins are prominent at the margins of the intestinal portal and continue on the ventral side of the fore-gut to meet at the posterior end of the heart, which is now a single tube, slightly bent toward the right. The neural folds are fused as far back as the eleventh somite, where the posterior neuropore is now known as the sinus rhomboidialis. The anterior neuropore is about to close, and in the head the neural tube shows three regions of dilation which represent the fore-brain, mid-brain, and hind-brain, respectively. The sides of the fore-brain are evaginating to produce the optic vesicles. In the hind-brain, five neuromeres can be identified. Twelve somites may be counted. The notochord extends forward to the fore-brain from the primitive streak which is now reduced to 0.3 mm.

Endodermal derivatives. — The fore-gut is in contact with the ectoderm ventrally, through the invagination of the stomodæum, so that the oral plate is composed of ectoderm and endoderm. At the sides, the walls of the fore-gut are fused to the ectoderm at points where the first visceral pouches (hyomandibular) will be located.

Mesodermal derivatives. — The somites now number twelve, and myocœles are still apparent. The mesomere is still unsegmented, but pronephric tubules have appeared in the region corresponding to somites 5–12. The four posterior tubules are growing back to form the pronephric duct. In the splanchnic mesoderm the blood islands are being converted into capillaries. The vitelline veins are prominent and continue forward into the heart, of which the endocardium and myocardium are distinct. The heart is supported by the dorsal mesocardium, the ventral mesocardium having disappeared. The primordial tubes, from the fusion of which the heart arose, continue forward as the ventral aortæ which bend around the pharynx (first aortic arches) and

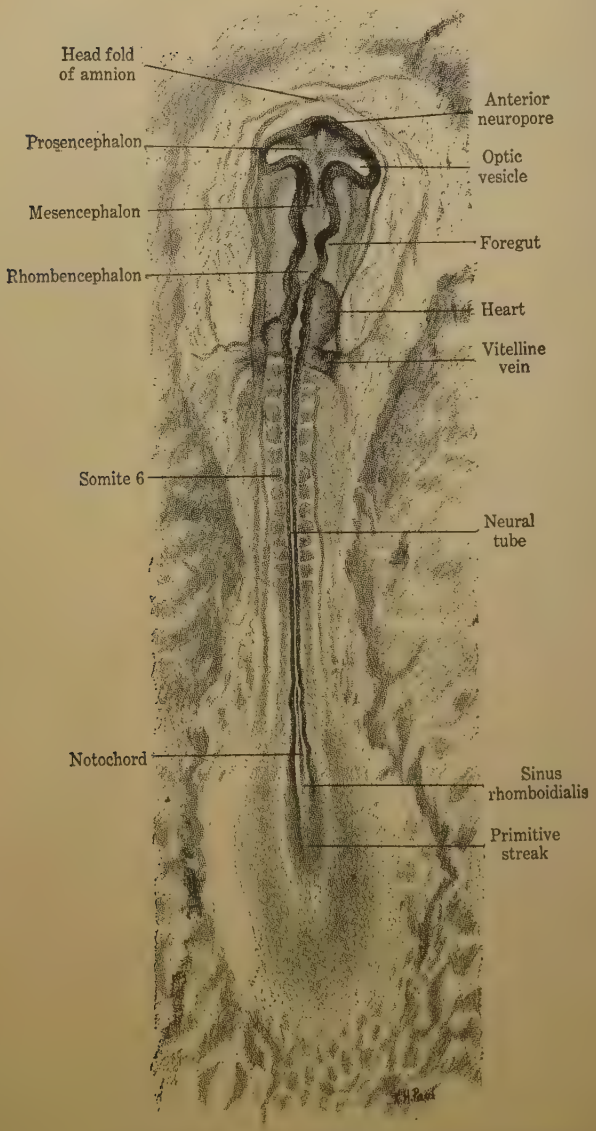

FIG. 162. — Dorsal view of 33 hour chick embryo. Cleared preparation. ×25.

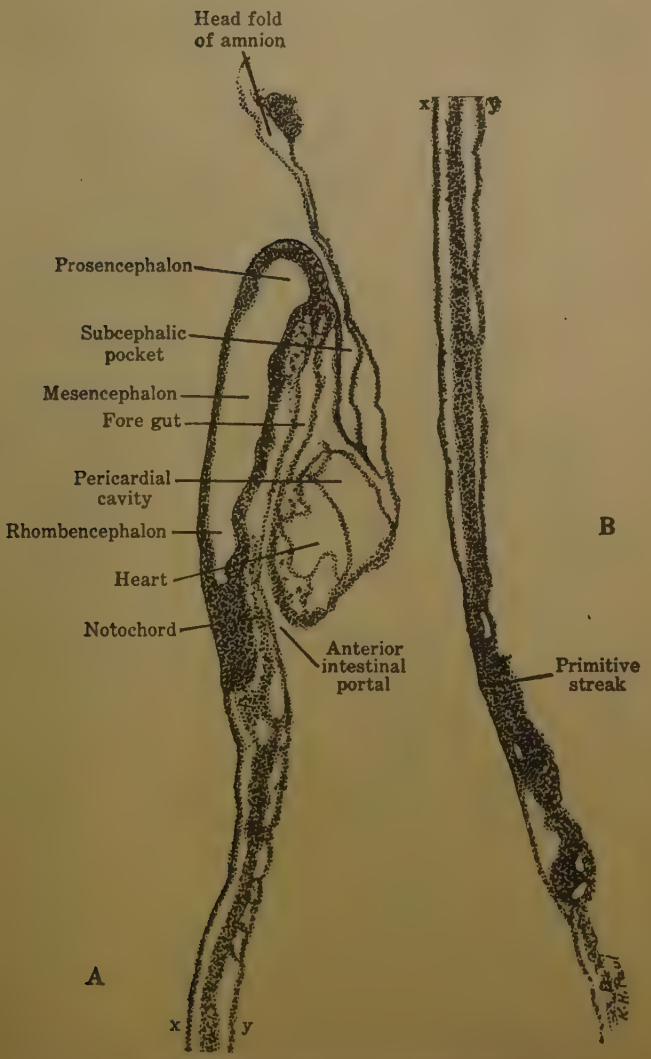

FIG. 163. — Sagittal section through 33 hour chick embryo. ×50.

continue backward along the dorsal surface of the pharynx as the dorsal aortæ. At the level of the primitive streak they are lost in a capillary nexus which foreshadows the vitelline arteries. From

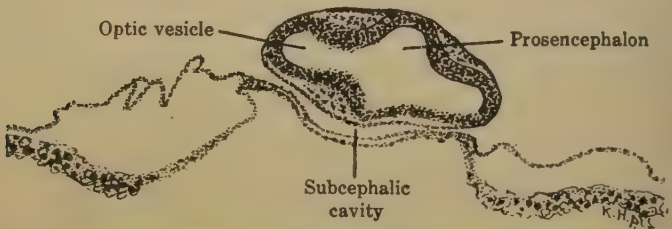

Fig. 164. — Transverse section through optic vesicles of 33 hour chick embryo. ×50.

a point immediately in front of the optic vesicle, the anterior cardinals course on either side of the neural tube, bending down to enter the heart with the vitelline veins. The notochord is slightly bent at the anterior end.

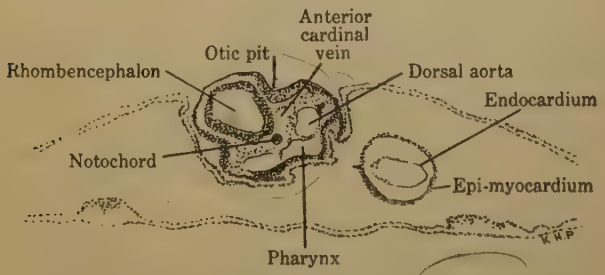

Fig. 165. — Transverse section through otic pits of 33 hour chick embryo. ×50.

Ectodermal derivatives. — The neural folds now extend to the eleventh somite and have fused throughout the length of the head. The anterior neuropore is almost closed. The three dilations which represent the prosencephalon, mesencephalon, and rhombencephalon are distinct. From the prosencephalon the two optic vesicles extend to the ectoderm of the sides of the head. Five neuromeres may be identified in the rhombencephalon. It has been asserted that in earlier stages three neuromeres may be identified in the prosencephalon and two in the

mesencephalon, while the first of the five noted above has resulted from the fusion of two original neuromeres destined to give rise to the metencephalon. At about this time a shallow

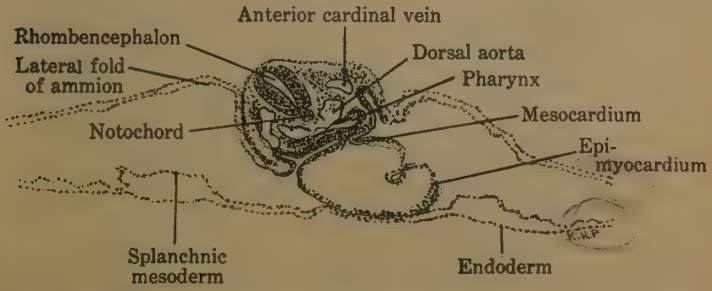

FIG. 166. — Transverse section through heart of 33 hour chick embryo. ×50.

depression in the floor of the prosencephalon, just in front of the tip of the notochord, marks the appearance of the infundibulum. The auditory placodes may sometimes be seen in sections as thickenings at the level of the constriction separating the last two neuromeres on either side.

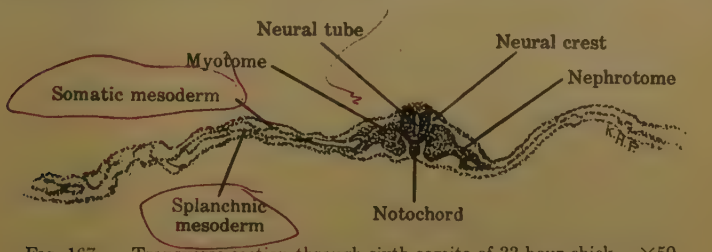

FIG. 167. — Transverse section through sixth somite of 33 hour chick. ×50.

C. THE FORTY-EIGHT HOUR STAGE

External form. — The chick at the end of the second day of incubation has usually attained a length of 7 mm., but the form of the body has been altered profoundly. As the head has been lifted away from the blastoderm, it has increased greatly in size and the cranial flexure, which was just appearing in the thirty-three hour chick, has become so pronounced that it is almost parallel with the hind-brain. With this growth and flexure the head is twisted, normally to the right, until it lies on one side, a

phenomenon known as **torsion.** At forty-eight hours, this torsion involves the chick as far back as the seventeenth somite. The posterior end of the chick lies in its original position, and at the extreme caudal end a tail fold is being formed. In the area vasculosa the capillaries have formed attachments with the vitelline arteries or veins, and at the border of this area is a circular vessel, the sinus terminalis. The fore-gut is now 1.4 mm.

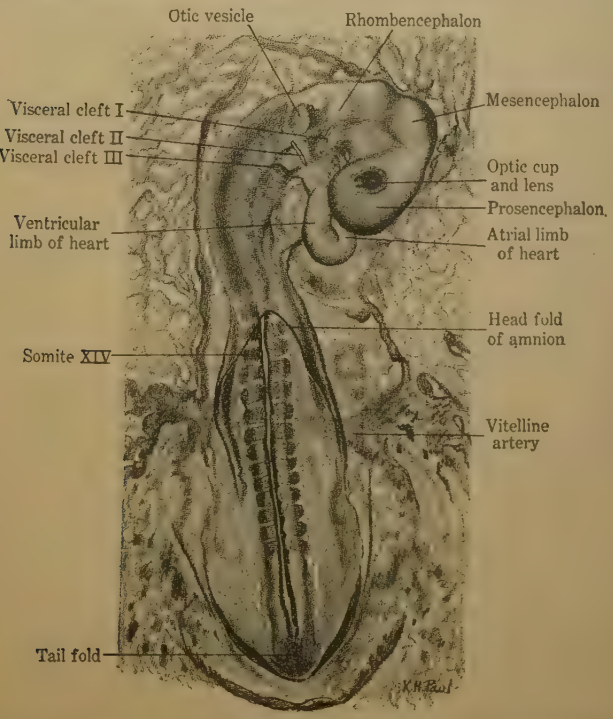

Fig. 168. — Dorsal view of 48 hour chick embryo. Cleared preparation. ×15.

in length, and the first of the three visceral pouches now communicates to the exterior following the rupture of the closing plate separating it from the corresponding visceral groove. The second and third visceral grooves are apparent, but their closing plates are still unperforated. In the visceral arches the first three aortic arches are apparent, arising from the ventral aorta. The

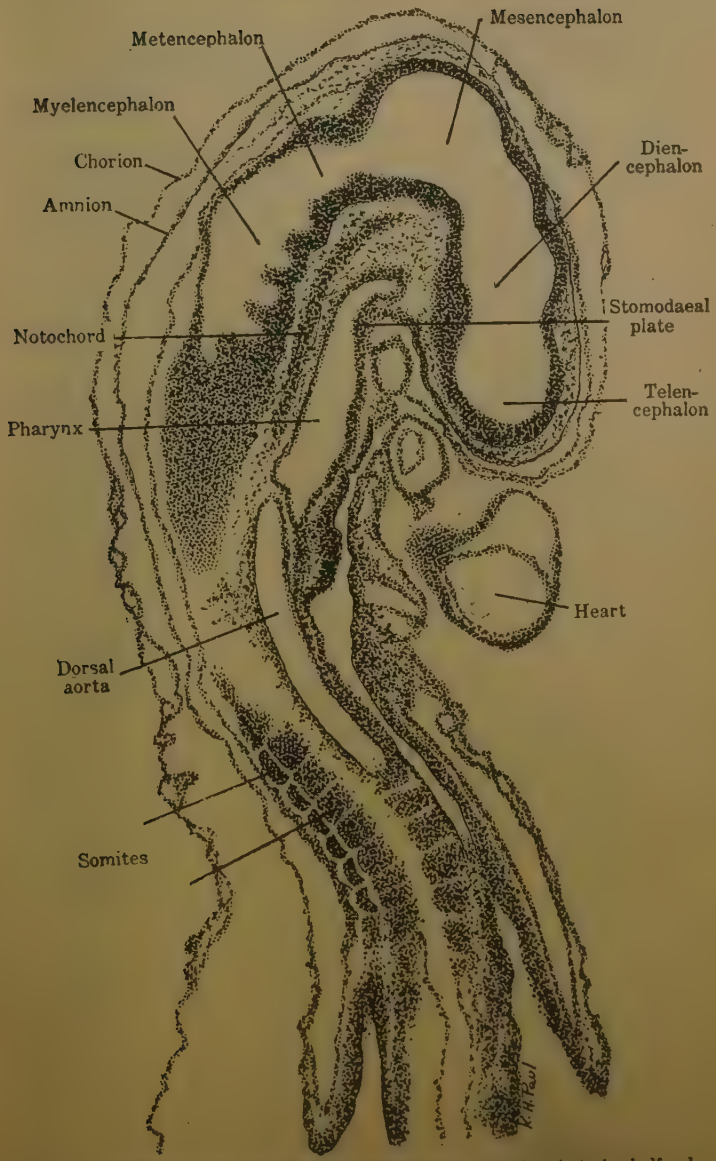

FIG. 169. — Sagittal section of 48 hour chick embryo. ×50. Anterior half only.

heart is now twisted so that the ventricular loop is uppermost. The vitelline veins are large and conspicuous, as are the vitelline arteries which leave the body at the level of the twenty-second somites. The neural tube is completely closed. In the head the five definitive regions of the brain are outlined, the prosencephalon having given rise to the telencephalon and diencephalon, and the rhombencephalon to the metencephalon and myelencephalon. The eye is now in the optic cup stage and the invagination of the optic vesicle continues down the stalk to form the chorioid fissure. The lens is in the form of a pit which has almost attained the vesicle stage. The ear is represented by an

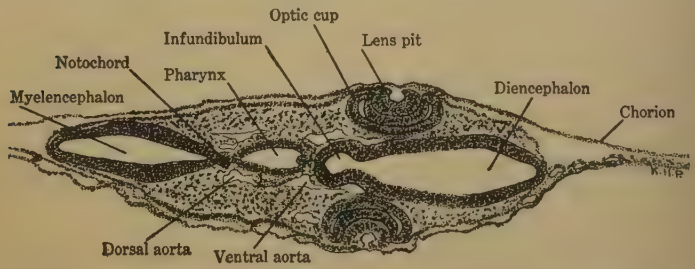

FIG. 170. — Transverse section through optic cup of 48 hour chick embryo. ×50.

otic vesicle which, owing to the cervical flexure, is almost on a level with the eye. There are twenty-seven somites at this stage. The primitive streak is found only in the tail fold. At this time the head fold of the amnion has grown back over the chick as far as the sixteenth somite.

Endodermal derivatives. — The stomodæum, invaginating from the surface of the ectoderm, has formed the oral membrane by contact with the fore-gut a little back of its most anterior point, so that there is a blind pocket in front of the oral plate, known as the preoral gut. Three visceral pouches are present, the first of which opens into the corresponding visceral furrow following the rupture of its closing membrane. The primordium of the thyreoid is represented by a ventral depression in the floor of the pharynx at the level of the second visceral pouches. The primordia of the lungs extend to the level of the sinus venosus. The mid-gut now has two shifting boundaries, the anterior intestinal portal and the posterior intestinal portal. The latter

is barely apparent as the opening of a shallow endodermal pocket in the tail fold which is the hind-gut.

Mesodermal derivatives. — The somites, twenty-seven in number, show a varying degree of specialization, with the most advanced at the anterior end. In these can be distinguished a loose aggregate of cells at the median ventral angle, the sclerotome, and a cap of epithelial cells at the lateral dorsal angle. Of this cap the cells nearest the epidermal ectoderm will become separated from the remainder to form the dermatome, while those nearest the neural tube will form the myotome.

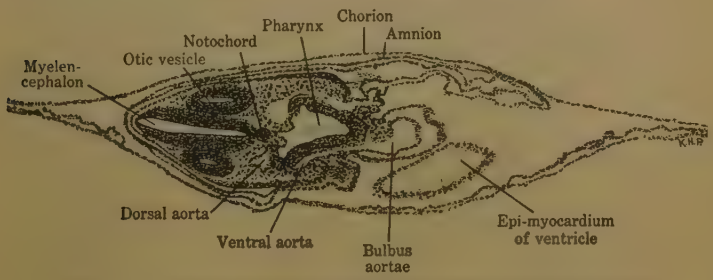

FIG. 171. — Transverse section through otic vesicles of 48 hour chick embryo. ×50.

The pronephric tubules in the more anterior somites have disappeared and mesonephric tubules are appearing in the mesomere posterior to the thirteenth somite. The pronephric (now the mesonephric) duct has acquired a lumen but has not yet attained its complete backward growth.

The heart is still tubular, but the ventricular limb of the cardiac loop has grown back and over the atrial limb so that the ventricular region is now caudal and dorsal with relation to the atrial region. Two aortic arches are present as a rule, and not infrequently the third has developed. From the first aortic arch a network of capillaries extends into the head. From these the carotid arteries will be formed. The dorsal aortæ have fused from a point back of the sixth somite as far as the level of the fifteenth somite. The vitelline arteries leave the dorsal aortæ at the level of the twenty-second somite but the aortæ continue back to the last somite. The vitelline veins are fused at their point of entrance into the heart as the sinus venosus. The an-

terior cardinals are prominent and extend from a capillary plexus in the head back toward the heart, where they are joined by the posterior cardinals and proceed as the common cardinals to enter the heart in the angles between the sinus venosus and the vitelline veins. The posterior cardinals may be traced back to the last somite. The heart of the chick commenced beating at the forty-fourth hour of incubation, so that the course of the blood is through the ventral aorta to the aortic arches and thence to the dorsal aorta. From the first aortic arch a network of capillaries supplies the head with blood (which is returned by way of the anterior cardinals). The main current of the stream passes down the dorsal aortæ to the point where these fuse to form the dorsal aorta. From the dorsal aorta, the somites are

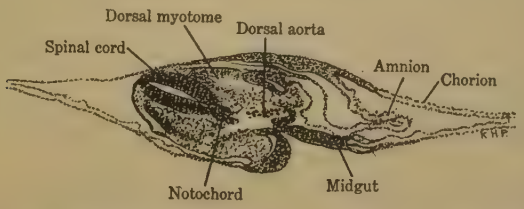

Fig. 172. — Transverse section through intestinal portal of 48 hour chick embryo. ×50.

supplied by capillaries, which will later become the intersegmental arteries. This blood is returned by way of the posterior cardinals. Leaving the dorsal aorta *via* the vitelline arteries, the blood passes through the capillaries of the area vasculosa to the sinus terminalis, and thence to the capillary drainage of the vitelline veins which return it to the heart.

The notochord is bent, not only at its tip (cranial flexure) but also at the point where the myelencephalon merges with the spinal cord (cervical flexure).

Ectodermal derivatives. — The brain now has acquired its five definitive vesicles. The telencephalon is enlarged but shows no particular differentiation. From the diencephalon project the constricted optic stalks which bear the optic cups with their inner sensory layer and outer pigmented layer. (The pigment will not arise until later.) The invagination by which the cups were formed continues down the stalk as the chorioid groove. On the

ventral surface of the diencephalon the infundibulum has deepened. Growing in toward it from the stomodæum is an ectodermal invagination, the hypophysis, which will fuse with the infundibulum to form the pituitary gland. The lens of the eye is in the pit stage, resulting from the invagination of a sensory placode. When the process is complete, the lens will be a vesicle completely withdrawn beneath the surface of the ectoderm, a condition which has already been attained by the otic vesicle,

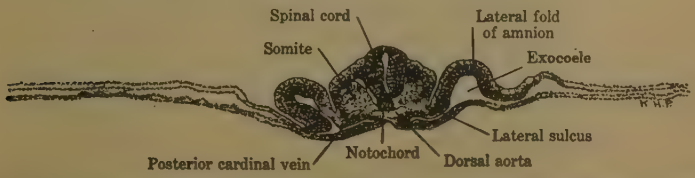

FIG. 173. — Transverse section through 14th somite of 48 hour chick embryo. ×50.

the primordium of the inner ear. Along the rhombencephalon and cord, the neural crest is to be seen as a narrow band of cells on each dorso-lateral angle.

D. THE SEVENTY-TWO HOUR STAGE

External form. — At the end of the third day of incubation, the total length of the embryo is 9.5 mm., but the curvature of the body is so great, on account of the cranial and cervical flexures in addition to the newly developed caudal flexure, that the greatest length, from neck to tail, is 7 mm. Torsion involves the body as far back as the vitelline arteries and will become complete during the fourth day. Anterior and posterior limb buds are now apparent at the levels of somites 17–19 and 26–32 respectively. The tail is curved forward. The fore-gut is still 1.4 mm. in length but has undergone further differentiation, indicated externally by the fact that the first three visceral clefts are open while the fourth is still interrupted by its closing plate. In the branchial arches four aortic arches may be seen. The telencephalon has given rise to the primordia of the cerebral hemispheres, and from the roof of the diencephalon, a small evagination represents the epiphysis or primordium of the pineal gland. The eye and ear, which were formerly in the same

transverse section, are now nearly in a cephalo-caudal relationship. The olfactory pits have made their appearance in the head. The semilunar (fifth cranial nerve), geniculo-acoustic (sev-

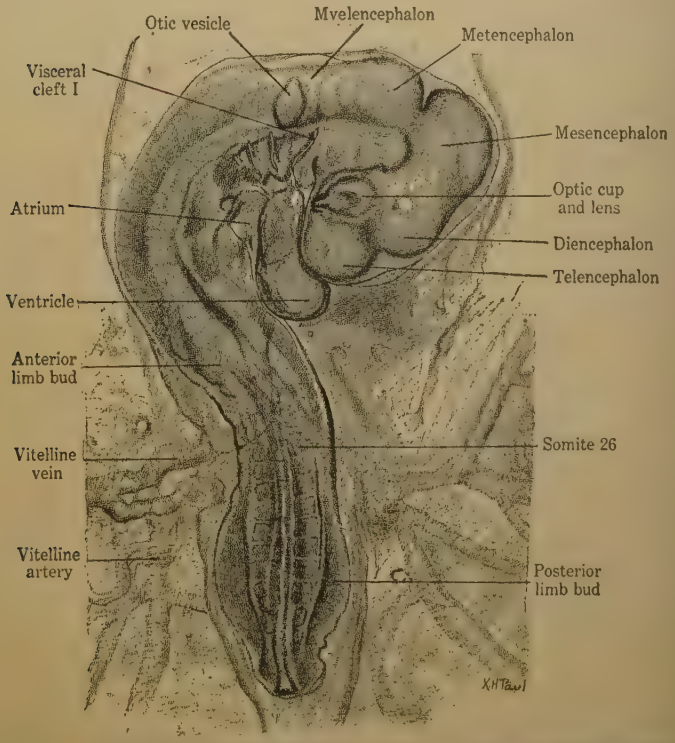

Fig. 174. — Lateral view of 72 hour chick embryo. Cleared preparation viewed from above. ×15.

enth, eighth) and petrosal (ninth) ganglia may be seen. There are approximately thirty-five somites. The primitive streak has disappeared. The amnion is completed by the fusion of head and tail folds. The allantois lies between the posterior limb buds.

Endodermal derivatives. — At the end of the third day the oral aperture has been formed by the rupture of the oral membrane separating the stomodæum and the fore-gut. Immediately anterior to this opening the preoral gut persists. The fore-gut

is still the same length as in the chick of forty-eight hours, but is more complex in structure. The thyreoid gland, which appeared

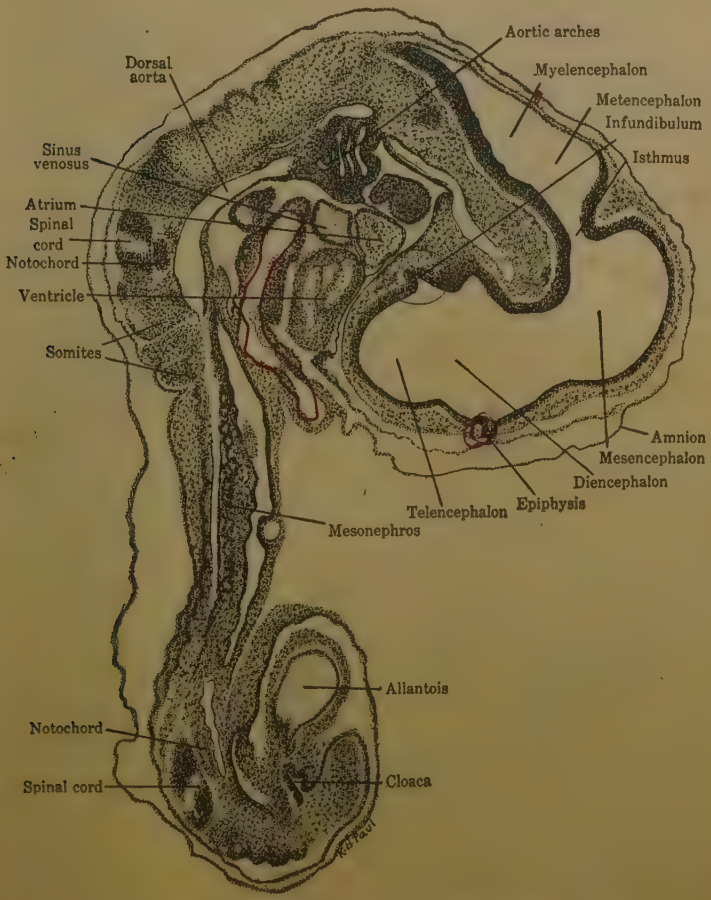

FIG. 175. — Sagittal section of 72 hour chick embryo. ×20.

during the second day, has now become differentiated into the distal dilation which will give rise to the gland proper and the thyreoglossal duct. The first three visceral pouches are open to the exterior, but the epithelial buds destined to give rise to the

thymus and parathyreoids are not yet apparent. The fourth visceral pouch is still separated from its branchial furrow by the closing plate. The laryngeo-tracheal groove has developed

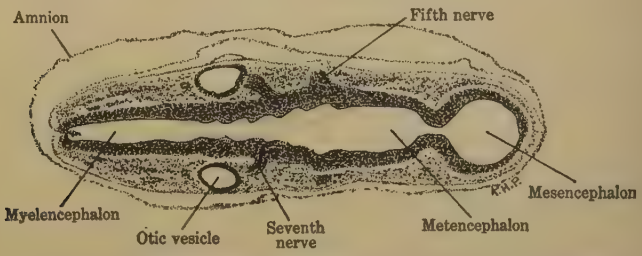

Fig. 176. — Transverse section through otic vesicles of 72 hour chick embryo. ×20.

in the floor of the pharynx just posterior to the fourth visceral pouches. At its posterior end the dorsal margins of this groove have closed together to form the primordium of the trachea which is thus set free from the œsophagus above. The trachea is

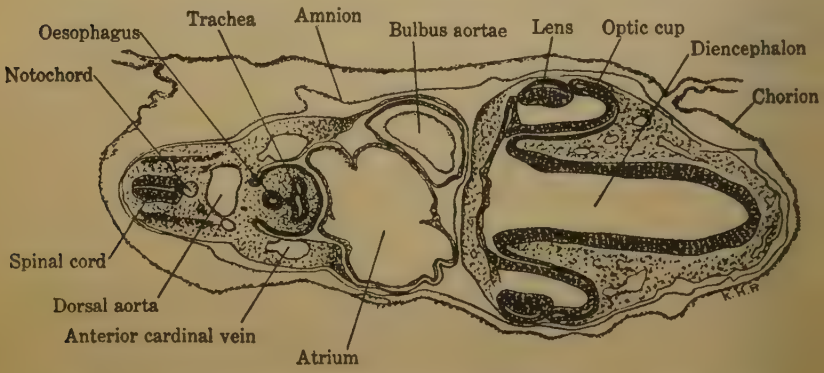

Fig. 177. — Transverse section through optic cup of 72 hour chick embryo. ×20.

bifurcated at the caudal end, thus giving rise to the two bronchial buds which are the primordia of the lungs.

The œsophagus, which is relatively narrow, is followed by a dilation which is to become the stomach. Posterior to this, the primordium of the liver may be seen as an evagination from the ventral floor of the duodenal region of the gut. The dorsal

pancreas arises from the duodenal region just dorsal to the liver at the end of the third day. The ventral primordia will not appear for another day.

The mid-gut region is gradually lessened by the advancing sulci which are cutting off the body of the embryo from the yolk. This region opens into the yolk-stalk which is still quite wide.

The hind-gut contained in the tail fold has not yet acquired its cloacal aperture nor has the proctodæum appeared. The floor of the hind-gut between the tail bud and the posterior intestinal portal evaginates to give rise to the allantoic primordium.

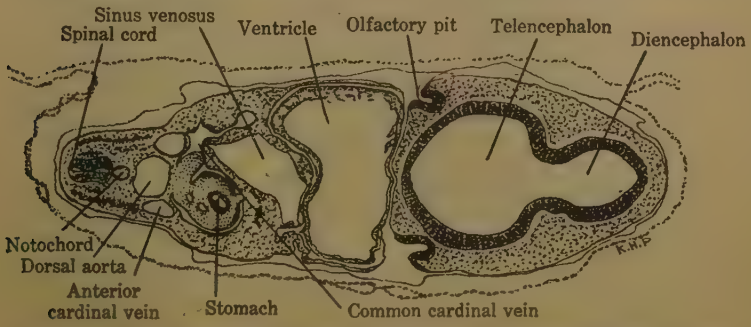

FIG. 178. — Transverse section through nasal pits of 72 hour chick embryo. ×20.

Mesodermal derivatives. — The somites, typically thirty-five in number, still show a varying degree of differentiation which is carried to its furthest point in the more anterior somites. The dermatome is now a thin sheet of cells along the dorso-lateral angle of the embryo, with the myotome parallel and internal; the sclerotome in these anterior segments is a large and loose aggregate of cells investing the neural tube, notochord, and aortæ.

The pronephric tubules have degenerated to a considerable extent but the nephrostomes opening into the cœlom may persist. The mesonephric tubules are now in process of development, with those in the more anterior segments most highly differentiated. The tubules between the thirteenth and thirtieth somites have progressed from the vesicle stage characteristic of those behind the twentieth somite, and some have ac-

quired a lumen and joined the pronephric duct which henceforward is known as the mesonephric duct. A few of the more anterior tubules develop nephrostomes, but these soon disappear.

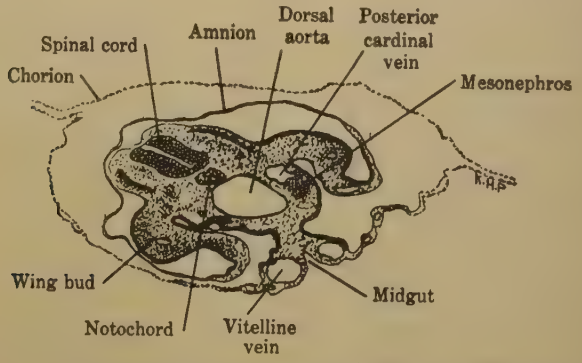

FIG. 179. — Transverse section through wing bud of 72 hour chick embryo. ×20.

Behind the twentieth somite, as far back as the thirtieth, only vesicles are formed. The mesonephric ducts have grown back and united with the cloaca.

The heart now shows a constriction between the atrial and ventricular region. Four aortic arches are developed of which

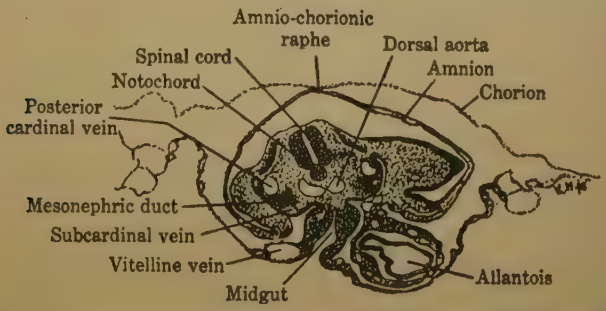

FIG. 180. — Transverse section through amnio-chorionic raphe of 72 hour chick embryo. ×20.

the first is becoming smaller, and sometimes has disappeared at this stage. The internal carotid arteries are now well developed, growing forward into the head from the point of union between the first arches and the dorsal aortæ. From the ventral end of

the first aortic arch the external carotid takes its origin. The pulmonary is sometimes apparent as a posterior prolongation of the ventral aorta at the point where the fifth arches will appear during the next twenty-four hours. The intersegmental arteries are now apparent as dorsal diverticula from the aorta between each pair of somites. The vitelline veins have fused for a short distance behind the sinus, thus giving rise to the meatus venosus. The anterior cardinal vein now possesses many branches from the head, among which are three intersegmental veins. The posterior cardinal has continued its backward growth dorsal to the mesonephric duct as far as the thirty-third somite. It

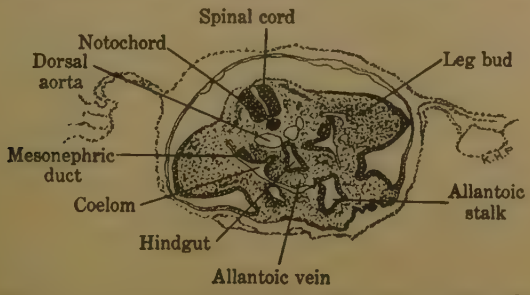

FIG. 181. — Transverse section through allantoic stalk of 72 hour chick embryo. ×20.

receives the intersegmental veins of this region. Where the posterior cardinals unite with the common cardinals, a capillary network indicates the beginnings of the allantoic veins.

Ectodermal derivatives. — The brain at the end of the third day has its five definitive vesicles even more sharply demarcated. From the telencephalon two lateral vesicles have evaginated to form the primordia of the cerebral hemispheres. In the diencephalon the epiphysis has appeared as a dorsal evagination. On the floor of this vesicle the infundibulum is almost in contact with the hypophysis. The mesencephalon is separated from the metencephalon by a deep constriction known as the isthmus. Along the sides of the myelencephalon may be distinguished the following cerebral ganglia: the semilunar of the fifth cranial nerve; the acoustico-facialis which will later separate into the geniculate ganglion of the seventh and the acoustic of the eighth; and the petrosal ganglion of the ninth. The eye has increased in

size and the lens is now free from the epidermal ectoderm. The ear, too, is in the vesicle stage and possesses a short endolymphatic duct, which has lost its connection with the epidermis. On the third day the primordium of the nose is represented by two olfactory pits anterior to the mouth.

REFERENCES

Duval, M. 1889. Atlas d'Embryologie.
Keibel and Abraham. 1900. Normaltafeln II, des Huhnes (Gallus domesticus).
Lillie, F. R. 1919. The Development of the Chick, 2nd Ed.
Patten, B. M. 1929. The Early Embryology of the Chick, 3rd Ed.

CHAPTER X

THE ANATOMY OF THE 10 MM. PIG EMBRYO

Pig embryos of from 10 to 12 mm. body length are particularly instructive for laboratory work in mammalian embryology as the size is sufficiently large for the study of external struc-

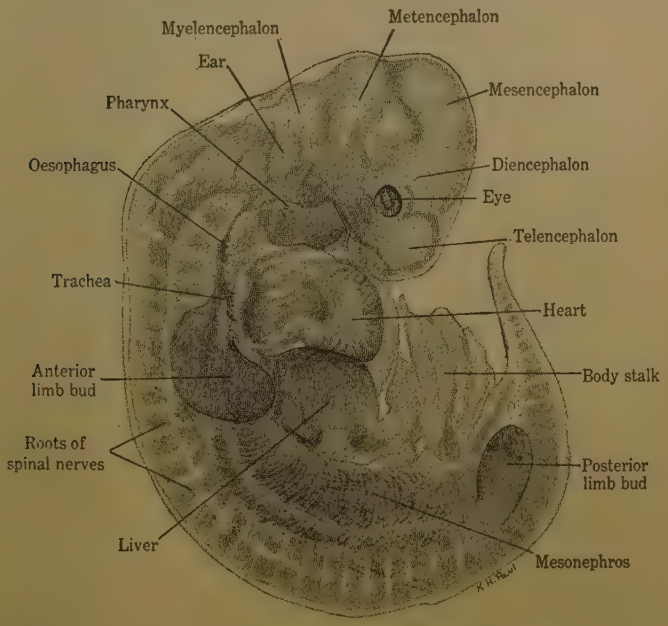

FIG. 182. — Lateral view of 10 mm. pig embryo. Cleared preparation. ×11.

tures and yet small enough to afford serial sections for a detailed study of the anatomy. The primordia of practically all the organ systems, excepting the skeleton and musculature, are present. In comparing the accounts given by different authors of this particular stage, it should be remembered that a large amount of

shrinkage takes place during the preparation of fresh sections, so that, as pointed out by Patten, an embryo of 12 mm. will not measure more than 9½ mm. when prepared for sectioning. The account which follows corresponds in general to the pig (*Sus scrofa*) of 10 mm. described by Keibel, of 12 mm. (Minot), 10 mm. (Prentiss), and 9.4 mm. (Patten).

External form. — The pig embryo at this stage is relatively more advanced than the chick of seventy-two hours. The body is sharply flexed, owing to the presence of the cranial, cervical, dorsal, and caudal flexures. In the head region the olfactory pits are well developed and are connected by the naso-lachrymal groove to a depression which surrounds the bulging eyeball. The five divisions of the brain are apparent through the relatively thin overlying epidermis. Four visceral grooves can be seen, the first of which, or hyomandibular, is the primordium of the external auditory meatus. The third and fourth grooves are compressed by the cervical flexure into a deeper depression known as the **cervical sinus.** A frontal view of the head shows the oral cavity bounded above by the frontal process in the middle, the maxillary processes at the side, while the lower jaw is represented by the mandibular arch.

In the trunk region, the buds of the pectoral and pelvic appendages are large but show no further differentiation. The contours of the somites, now 44 in number, are apparent along the back, and ventral to these can be seen the outlines of the heart, liver, and mesonephros. In some specimens there appears between the limb buds a thickened ridge from which the mammary glands develop and which is hence known as the milk line. The umbilical cord projects from the ventral side of the embryo. Between this and the base of the slender tail is a small protuberance, the **genital tubercle,** or primordium of the external genitalia.

Endodermal derivatives. — The preoral gut still persists anterior to the oral aperture. Ventral to this and seen best in sagittal section, is the long and slender hypophysis, now in contact with the infundibulum of the diencephalon. Both the hypophysis and infundibulum, it should be remembered, are of ectodermal origin. The pharynx is dorso-ventrally compressed, and from its floor the tongue is arising. Four visceral pouches

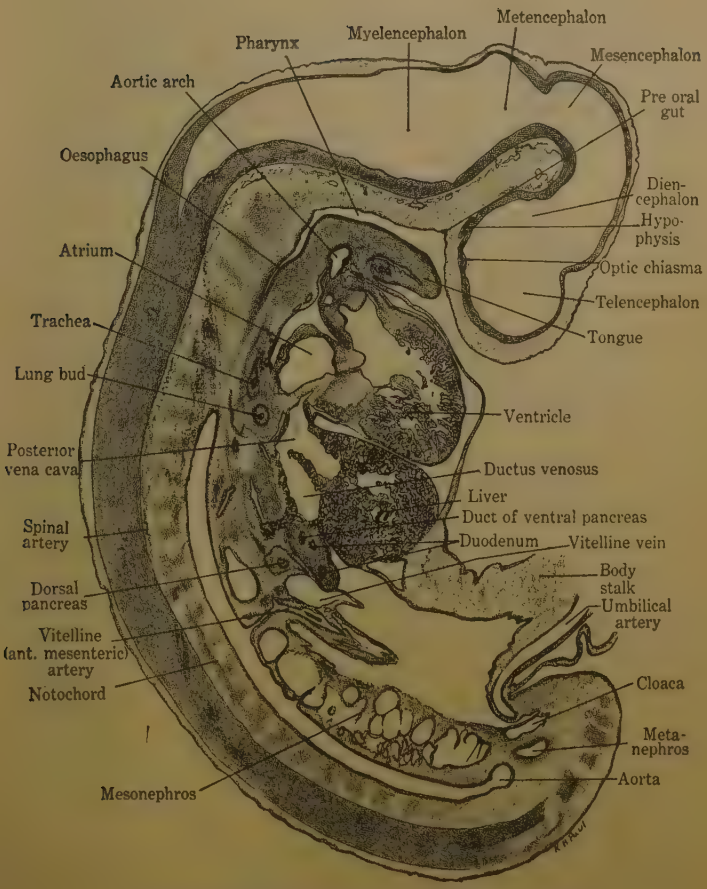

FIG. 183. — Sagittal section of 10 mm. pig embryo. ×16¾.

are present, corresponding to the visceral grooves already noted. These do not unite to become visceral clefts but remain separated by their closing membranes. Between the second and third pouches the thyreoid gland appears. From the level of the fourth pouch a short laryngeal groove is prolonged into the trachea which has given rise to the bronchial buds, three in number. Two of these, the **primary bronchi,** have arisen by the bifurcation of

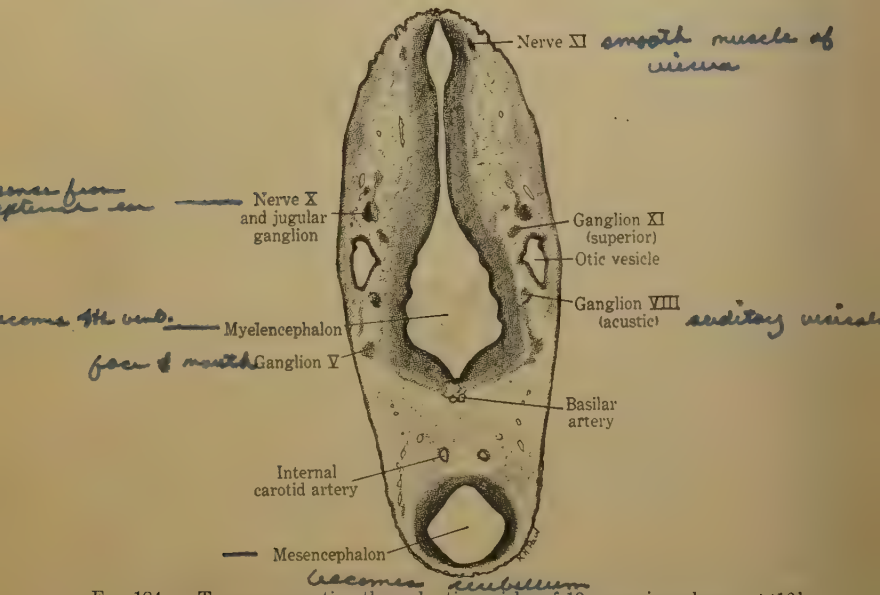

FIG. 184. — Transverse section through otic vesicles of 10 mm. pig embryo. ×16½.

the trachea; the third or **apical bud,** which will give rise to the **eparterial bronchus,** develops anterior to the right primary bronchus. The œsophagus is relatively long and narrow and, just posterior to the level of the lung buds, passes into the stomach which is dilated and shows a slight dorsal curvature. Posterior to the stomach the duodenal glands, liver and pancreas, are well developed. The liver, now a large glandular mass traversed by the capillaries of the hepato-portal veins, retains its original connection with the duodenum as the ductus choledochus from the distal end of which the gall-bladder is forming. Both dorsal

and ventral primordia of the pancreas are present, the duct of the latter arising from the ductus choledochus. The long and slender intestine extends into the umbilical cord as the intestinal loop, to the base of which the yolk stalk is still attached. Just posterior to this, a slight enlargement may sometimes be observed which indicates the boundary between the large and small intestine. The hind-gut is divided into a dorsal rectum and ven-

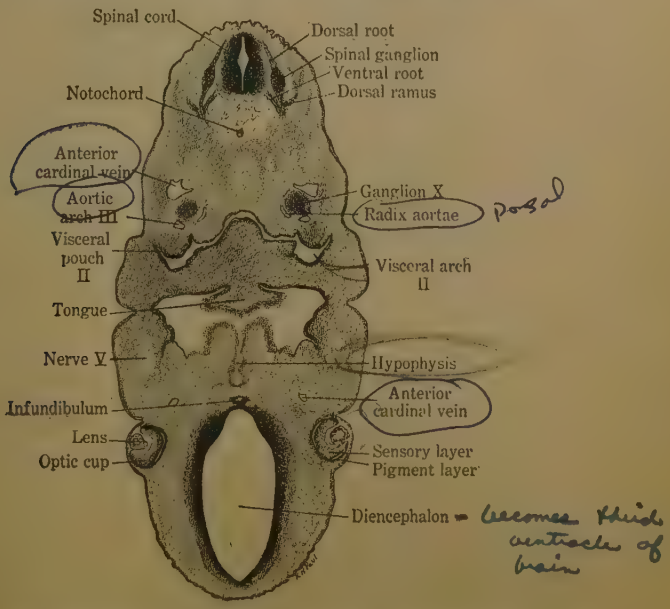

FIG. 185. — Transverse section through optic cups of 10 mm. pig embryo. ×16½.

tral urogenital sinus, prolonged into the allantoic stalk. The sinus and rectum unite in a common cloaca which has not yet established connection with the proctodæum. Immediately posterior to the cloacal plate, a small blind pocket represents the postcloacal gut.

Mesodermal derivatives. — The notochord extends from the vicinity of the floor of the mesencephalon into the tail, following the flexures of the body.

The somites have long since become differentiated into the

myotome, dermatome, and sclerotome. In the tail region, the sclerotomes are separated into the cranial and caudal arcualia from which the vertebræ will originate.

In the pig of 10 mm., the pronephric stage has been passed; the mesonephros is at the height of its development, forming a great Wolffian body with its complicated network of interwoven tubules; while the mesonephric duct (originally the pronephric

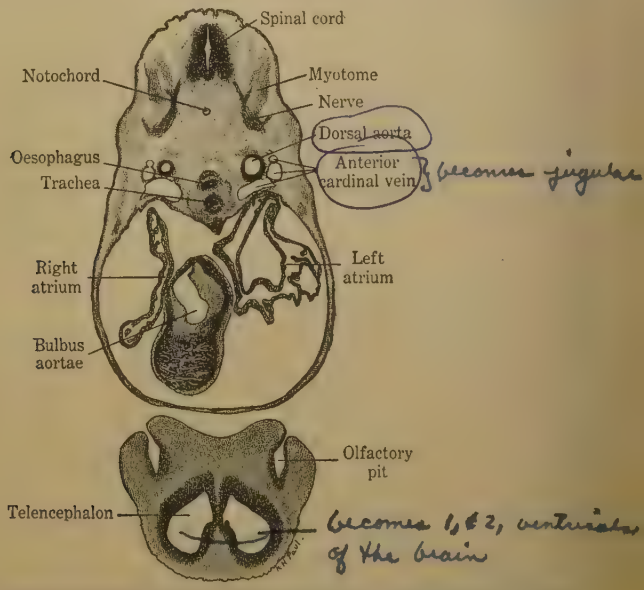

FIG. 186. — Transverse section through olfactory pits of 10 mm. pig embryo. ×16½.

duct) may be recognized along the ventral margin. Emerging from the mesonephroi, the ducts enter the urogenital sinus at the same level as the allantoic stalk. From each duct a narrow stalk runs dorsally and forward as the metanephric duct, or ureter, which at its distal end is enlarged to form the pelvis of the metanephros. Around the pelvis the posterior portion of the nephrotomal band will produce the secretory tubules of the definitive kidney at a later stage. On the median ventral margins of the mesonephroi are swellings which constitute the genital ridges, primordia of the gonads. The cœlom is partially divided into

the pericardial and abdominal cavities by the septum transversum. The mesenteries of the principal viscera are in evidence. The liver is still suspended in the ventral mesentery. A dorsal mesocardium is present.

The heart of the 10 mm. pig has the four main chambers established, although not yet completely separated into right and left halves. The sinus venosus now enters the right atrium through

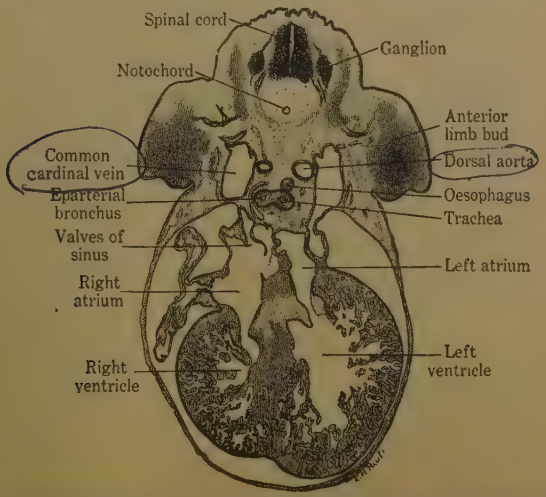

FIG. 187. — Transverse section through sinus venosus of 10 mm. pig embryo. ×16½.

a slit guarded by the valves of the sinus. The right and left atria are partially separated by the interatrial septum in which can be seen an opening, the **foramen ovale.** The atrio-ventricular canal leading to the ventricle is partially separated into right and left halves by the endocardial cushion. The ventricle is partially divided by the interventricular septum. The bulbus arteriosus is still undivided. From the ventral aorta three aortic arches curve around the pharynx to unite with the dorsal aorta. These are the third, fourth, and sixth aortic arches; the first and second have degenerated, while the fifth seldom appears as a separate structure. The pulmonary arteries are growing back from the sixth aortic arches.

As prolongations of the original ventral and dorsal aortæ,

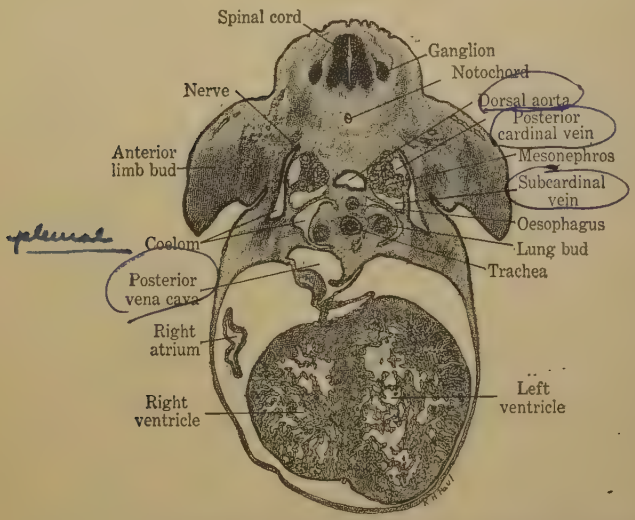

FIG. 188. — Transverse section through lung buds of 10 mm. pig embryo. ×16½.

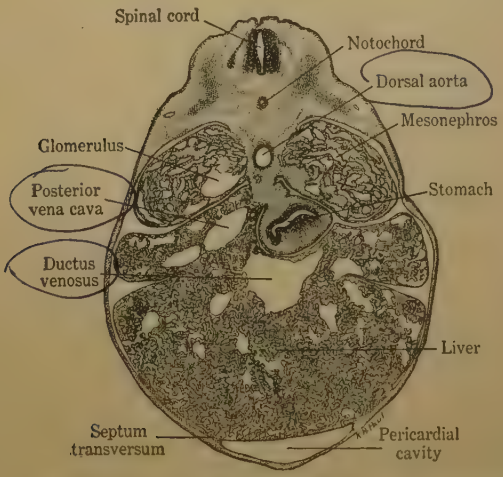

FIG. 189. — Transverse section through stomach of 10 mm. pig embryo. ×16½.

the external and internal carotid arteries, respectively, run forward into the head. The internal carotid arteries are united at the level of the isthmus between the mesencephalon and the metencephalon with the basilar artery, which serves to unite them with the vertebral arteries, arising from the anastomosis of intersegmental arteries in the cervical region. At the 10 mm. stage the vertebral arteries have lost their intersegmental con-

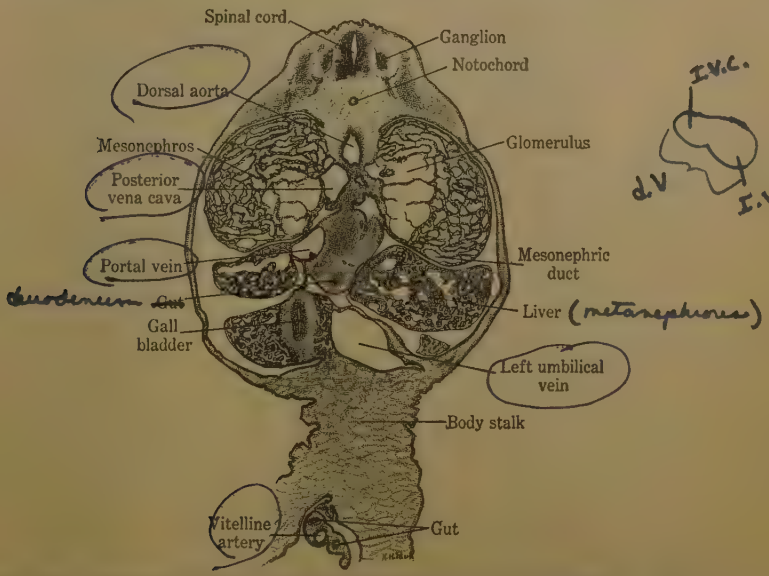

FIG. 190. — Transverse section through gall bladder of 10 mm. pig embryo. ×16½.

nections with the aorta except at the posterior end, where the seventh cervical intersegmental artery persists and grows out into the pectoral limb bud to form the subclavian artery. Near the point of origin of the subclavian, the dorsal aortæ are fused and run back as a single median aorta into the tail. Dorsally, branches are given off from the aorta as intersegmental arteries of the trunk. Laterally, many small branches supply the glomeruli of the mesonephros. Ventrally, the dorsal aorta gives off the cœliac artery and anterior mesenteric arteries to the gut. Two large umbilical (allantoic) arteries run from the dorsal aorta

into the umbilical cord. The aorta continues into the tail as a relatively slender vessel, the caudal artery.

The vitelline veins are much smaller than in the chick of seventy-two hours, for the yolk sac from which they drew their blood is nearly degenerated. In the pig at this stage they drain the gut area and converge towards the liver where they unite to

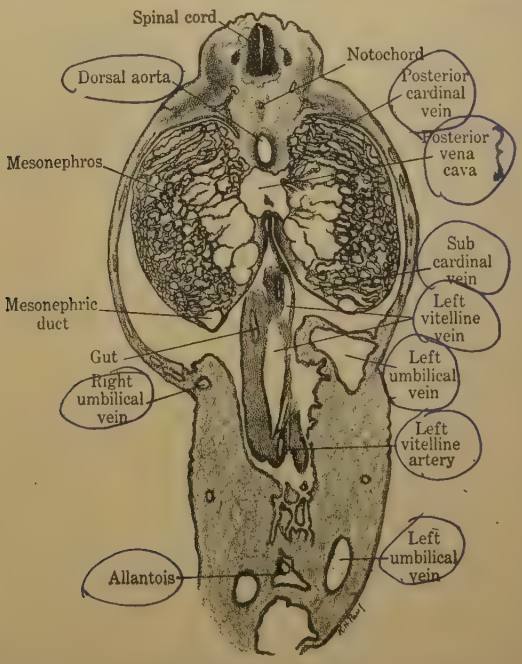

FIG. 191. — Transverse section through intestinal loop of 10 mm. pig embryo. ×16¼.

become the portal vein. Within the liver they are broken up into capillaries which emerge as the hepatic veins to the sinus venosus. Of the somatic veins, the anterior cardinals are still prominent and are joined by an extensive series of head veins. In the cervical region the anterior cardinals receive the dorsal intersegmental veins as well as the external jugular from the mandible. As the anterior cardinals enter the common cardinal veins, they are joined by the posterior cardinals, which have

already lost part of their drainage area to the subcardinal veins passing through the ventral portions of the mesonephroi. Numerous small venous channels serve to connect the subcardinals and postcardinals during this period. The posterior vena cava has already made its appearance as a direct connection from the subcardinals to the liver. The umbilical (allantoic) veins proceed from the allantois toward the heart but are now surrounded by the liver, within which they are, like the vitelline veins, breaking up into capillaries. The left umbilical maintains its original channel, now known as the ductus venosus.

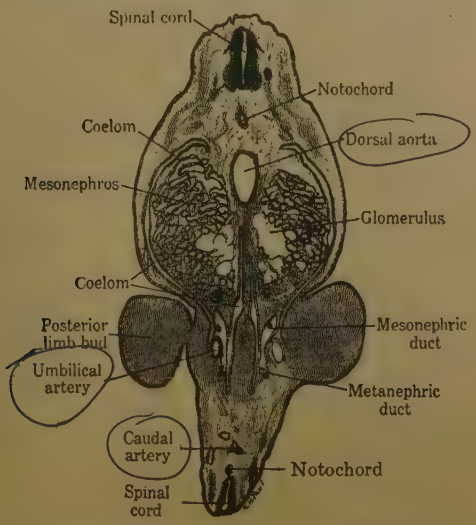

FIG. 192. — Transverse section through metanephric duct of 10 mm. pig embryo. ×16½.

Ectodermal derivatives. — The epidermal derivatives of the ectoderm have already been enumerated in the description of external form. There remain for consideration the nervous system and sense organs. The five definitive vesicles of the brain are well marked. From the telencephalon arise the two lateral cerebral vesicles. This division of the brain is separated from the diencephalon by two points of reference, the optic recess in the floor, and the velum transversum in the roof. From the

diencephalon spring the optic stalks, leading to the optic cups, and the infundibulum, now in contact with the hypophysis as mentioned above. The posterior boundary of the diencephalon is indicated by the tuberculum posterius arising from the brain

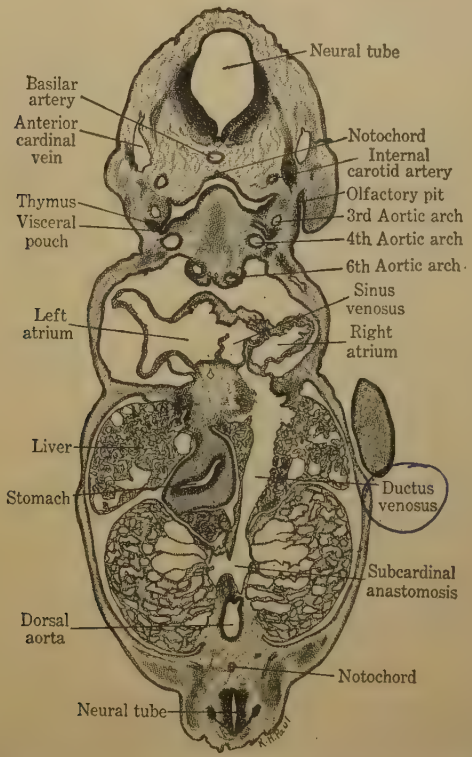

FIG. 193. — Frontal section through 10 mm. pig embryo, at level of ductus venosus. ×16½.

floor. The epiphysis seldom appears at this stage. The mesencephalon, with the third cranial nerve arising from its floor, is demarcated at its posterior end by the deep constriction of the isthmus. The metencephalon is distinguished from the myelencephalon by its thicker roof. From the isthmus the fourth cranial nerve runs forward, like the third, to the mass of meso-

derm surrounding the eyeball, from which the eyeball muscles will be formed. Conspicuous at the ventro-lateral margin of the metencephalon is the large semilunar ganglion of the fifth cranial nerve. From the floor of the myelencephalon, the sixth cranial nerve emerges to run forward toward the eye. Immediately following this, the geniculate ganglion of the seventh and the acoustic ganglion of the eighth are in close connection. The ninth cranial nerve has two ganglia, the dorsal superior ganglion and ventral petrosal, while the tenth similarly possesses a dorsal jugular and ventral nodose ganglion. The eleventh cranial nerve possesses at this stage a small ganglion (**of Froriep**) which disappears in the adult. The last of the cranial nerves, the twelfth, arises from the floor of the myelencephalon by a number of small roots and without a ganglion. In the region of the spinal cord the segmental nerves arise from the cord by two roots, of which the dorsal is associated with a spinal ganglion. The trunk is very short and soon divides into three main branches. The dorsal and ventral rami run to these respective regions of the body wall, while the third, or ramus intercommunicans, unites the spinal nerve with a ganglion of the sympathetic chain.

The nose is now represented by the olfactory pits. The eye is in the optic cup stage with a well-marked chorioid fissure and groove, while the lens is completely separated from the outer ectoderm and is now in the vesicle stage. Of the various regions of the ear, all the primordia are now established. The otic vesicle with its endolymphatic duct, representing the inner ear, is in close juxtaposition to the first visceral (hyomandibular pouch) which will give rise to the auditory tube and chamber of the middle ear; the external auditory meatus, or outer ear, will arise from the first or hyomandibular groove.

REFERENCES

Arey, L. B. 1924. Developmental Anatomy.
Keibel, F. 1897. Normaltafeln, I, des Schweines (Sus scrofa domesticus).
Lewis, F. T. 1902. The gross anatomy of a 12 mm. pig, Am. Jour. Anat., Vol. 2, pp. 211–226.
Minot, C. S. 1911. A Laboratory Textbook of Embryology, 2nd Ed.
Patten, B. M. 1927. The Embryology of the Pig.
Wallin, E. 1917. A teaching model of a 10 mm. pig embryo, Anat. Rec., Vol. 5, pp. 17–45.

PART IV

MICROSCOPICAL TECHNIQUE

CHAPTER XI

PREPARATION OF EMBRYOLOGICAL MATERIAL

A method much employed in the study of comparative embryology is that of cutting a preserved egg or embryo into a series of extremely thin slices, and arranging these in order upon a glass slide, so that they may be examined under the microscope. This method came into vogue on account of the small size of the objects to be studied and of the limitations of the microscope. The older embryologists, however, were limited to the study of entire embryos and of minute dissections. These methods are still of great value in supplementing the study of serial sections, for it is a difficult mental exercise to translate sections into terms of the whole embryo. The single section, especially, is meaningless except when interpreted as a part of the complete series. It is very helpful, therefore, when facilities permit, for each student to prepare for himself a whole mount and a series of sections through one of the embryos he is to study.

A. COLLECTION AND REARING OF EMBRYOS

While it is true that preserved embryos of the more important laboratory types may be obtained from the biological supply houses, it is often desirable to collect and rear live embryos.

The frog. — There are some sixty species of tailless amphibia within the continental limits of the United States. Although the capture of adults in a pond where eggs are found is strong circumstantial evidence as to the species of the eggs, even this evidence is often lacking, so that the ability to identify the eggs or larvæ from their own characteristics is highly desirable. A key to the eggs and larvæ of some of the common Eastern frogs

and toads is found in Wright's "Life History of the Anura of Ithaca, N. Y." For the Pacific slope fauna, see Storer, "A Synopsis of the Amphibia of California." The eggs of the salamander, Ambystoma, are laid at the same time and in the same localities as those of the early frogs, but may be distinguished from them by the greater proportion of jelly to the eggs in the mass of spawn.

The rate of development of the frog's egg depends upon the temperature of the water. In the laboratory, the eggs will hatch in about one week after laying, at the ordinary room temperature. The egg masses should be kept in clean glass containers with at least ten times as much water. The water should not be changed until after hatching, when the larvæ should be transferred to fresh water with aquatic plants. After the assumption of the tadpole form, they should be fed small pieces of finely ground meat. Metamorphosis may be hastened by feeding fresh or desiccated thyreoid tissue.

Artificial fertilization is the best method of obtaining the earliest stages of development. The testes and vasa deferentia of the male are teased out in a watch glass of water. The eggs from the distal portions of the oviducts are placed in this water for five minutes and then removed to glass containers with not more than four inches of water.

The chick. — In collecting hens' eggs for incubation, it is a truism that they must be fresh and fertile. The best results are obtained from trap-nested eggs in the spring semester. The egg is normally laid in the gastrula stage (Chapter III), but in those cases where the egg does not reach the distal end of the oviduct by 4 P.M., it is retained till the following morning and undergoes further development. After laying, the egg cools and development ceases until incubation is commenced. The fertilized egg is viable for five weeks at a temperature of 8°–10° C. The time of hatching, as in the frog's egg, is dependent upon the temperature. The minimum temperature at which development will take place is about 25° C.; the optimum is 37° C., at which temperature the egg will hatch in twenty-one days; the maximum temperature is about 41° C. In incubating eggs, care must be taken to keep the air in the incubator moist and to rotate the eggs once a day.

Instructive demonstrations may be made by opening the shell and shell membranes under aseptic conditions and removing a bit of the albumen. A window of celloidin placed over the opening and carefully sealed will permit of observations on the development of the embryo for several days. An alternative method is that of opening the egg and placing the contents in a sterilized small Stender dish. A glass ring is placed on the yolk to keep it beneath the surface of the albumen, and the dish is covered and placed in the incubator. If this operation is carried on under aseptic conditions, development will continue for two or three days.

The mammal. — The early stages of development in any mammal are valuable. The larger embryos are visible as protuberances on the inner side of the uterine tubes. The tube should be slit open and the embryos exposed by cutting open the embryonic membranes which surround them. Smaller stages are obtained by washing out the contents of the tube with normal salt solution or preserving it entire.

The pig. — Pig embryos may be obtained in quantities from any good-sized packing house. As many as eighteen may be found in a single female, but the average number is eight. The period of gestation in the pig is 121 days. Pig embryos of 10 mm. body length are the most useful in the elementary course. Later stages are of value in the detailed study of organogeny.

B. PRESERVATION OF MATERIAL

The preliminary preparation of material for microscopical work involves three distinct operations: **killing, fixing,** and **preservation.** In practice, two or three of these operations are performed by a single reagent known as a "**fixing fluid.**" Such a reagent should kill the embryo so rapidly that it will undergo the minimum of post-mortem changes; it should preserve the structures of the embryo with as life-like an appearance as possible; and it should harden the soft parts so that they may undergo the later processes of technique without loss of form or structure. Some fixing fluids, such as alcohol or formalin, may be used indefinitely as preservatives, but the majority are used for a particular optimum period, and then washed out and replaced by alcohol.

The frog. — The frog's egg, *before* hatching, is best fixed by **Smith's fluid.**

Potassium bichromate	0.5 gram
Glacial acetic acid	2.5 cc.
Formalin	10.0 cc.
Distilled water	75.0 cc.

1. Cut the egg masses into small pieces of about twenty-five eggs each, and submerge them in a dish of Smith's fluid for twenty-four hours. A quantity equal to ten times the volume of the eggs should be used.

2. Rinse the eggs in water and wash with a 5 per cent aqueous solution of formalin until no more free color comes out. The eggs may be kept indefinitely in this fluid. If it is desired to remove the egg membranes, proceed as follows:

3. Wash in water for twenty-four hours, changing the water several times.

4. Place the eggs in **Eau de Javelle,** diluted with three times its volume of water, and shake gently from time to time during a period of from 15 to 30 minutes until the membranes are almost dissolved and will shake off.

5. Rinse in water and run through 50 per cent and 70 per cent alcohol, an hour to a day each, and preserve in 80 per cent alcohol.

After hatching, larvæ are best fixed in **Bouin's fluid.**

Picric acid, saturated aqueous solution	75 cc.
Formalin	25 cc.
Glacial acetic acid	5 cc.

1. Larvæ are left in this fluid from one to eighteen hours, according to size.

2. After rinsing in 50 per cent alcohol, wash in 70 per cent alcohol, to which has been added a few drops of lithium carbonate, saturated aqueous solution, until the yellow color is extracted, and preserve in 80 per cent alcohol.

The chick. — The chick embryo must be removed from the shell, albumen, and yolk before fixation. As the early stages are more difficult to handle, it is advisable to practice this operation on embryos of seventy-two hours' incubation and then work backward toward the stages of the first day.

1. Place the egg in a dish 3 inches high and 6 inches in diameter, two-thirds full of **normal saline solution,** warmed to 40° C.

Sodium chloride	0.75 gr.
Water	100.00 cc.

2. Crack the shell at the broad end with the flat of the scalpel, and pick away the pieces of shell until an opening slightly larger than a half dollar has been made. Remove the outer and inner shell membranes. Invert egg beneath the surface of the salt solution and allow the contents to flow out. The blastoderm, containing the embryo, will rotate until it is uppermost. With fine-pointed scissors, cut rapidly a circle of blastoderm, about the size of a quarter, with the embryo at the center. With blunted forceps, pull the blastoderm and adherent vitelline membrane away from the yolk and albumen, waving it gently beneath the surface of the salt solution to remove all yolk.

3. Submerge a Syracuse watch glass in the salt solution and float the embryo into this. Remove the watch glass carefully from the large dish and examine the embryo with a dissecting lens. If the vitelline membrane has not yet separated from the blastoderm, it should be removed at this time with fine-pointed forceps and needles. Make sure that the embryo lies dorsal side up, as it did when the egg was opened.

4. Slide a cover glass under the embryo, and remove all salt solution with a pipette, taking care that the embryo lies in the center of the cover glass. Lift the cover glass by one corner so that the overhanging edges of the blastoderm fold under, and place it in a dry watch glass, where it is to remain until the edges of the blastoderm *only* are dry. While the embryo is becoming attached to the cover glass, remove the yolk, albumen, and pieces of shell from the dish of salt solution to a slop jar, reheat the salt solution to 40° C. and prepare another embryo. Three embryos of each stage are to be prepared.

5. When the edges of the blastoderm are dry, add a few drops of Bouin's fluid from a pipette held just over the center of the embryo. After five minutes, drop the cover glass, embryo side up, into a small Stender dish of Bouin's fluid and leave from two to four hours.

6. Rinse in 50 per cent alcohol, wash for two days in 70 per

cent alcohol to which lithium carbonate has been added or until the yellow color is extracted from the embryo, and preserve in 80 per cent alcohol.

The pig. — Embryos of 6 mm. body length and over are easily located in the uterine wall. Slit open the uterus and remove the embryo with fine-pointed forceps and a horn spoon, taking pains not to rupture the membranes. Place at once in Bouin's fluid. Embryos of 10 mm. body length should be fixed for four hours. Rinsing and preserving are done as for the frog or chick. Larger embryos should have the body cavity slit open to admit the fixing fluid. Fœtal pigs of 6 inches or more should be injected through the umbilical artery with formalin (20 per cent aqueous solution). This solution is also injected into the body cavity and cranium, after which the fœtus is submerged in the same medium for a week and preserved in 6 per cent formalin.

C. WHOLE MOUNTS

It is very helpful to have some embryos mounted entire for comparison with the serial sections. In making these whole mounts, the embryos are **stained, cleared,** and **mounted,** i.e., transferred to a final medium for preservation and examination on the slide beneath a cover glass.

The frog. — Frog eggs and embryos may be mounted as opaque objects with the natural pigmentation, or they may be cleared and stained as transparent mounts.

Opaque mounts. —

1. Prepare a saturated aqueous solution of thymol. Filter the solution and add gelatin until saturated. Remove the supernatant liquid.

2. Liquefy the gelatin by immersing a small quantity, in a test tube, in a dish of hot water. Fill a hollow-ground depression slide with gelatin and allow to cool.

3. With a hot needle, melt a small hole in the gelatin, sufficiently large to hold the embryo. Place the embryo in the desired position and hold it in place until the gelatin has cooled.

4. Add a drop of gelatin just warm enough to be liquid and cover with a cover glass which has been slightly warmed. When the gelatin has cooled, any surplus may be removed from the

edges of the cover glass with a toothpick wrapped in moist cotton. In order to prevent the later formation of bubbles, the edges of the cover glass should be painted with gold size or Valspar.

Free-hand sections and dissections are admirably mounted by this method, but great care must be exercised to prevent the formation of air bubbles through cracks in the gold size.

Transparent stained mounts. —

1. Bleach the embryo until white, in hydrogen peroxide. About one week is required for this purpose. Embryos that have been preserved in 80 per cent alcohol should first be passed through 70 and 50 per cent alcohol to water, an hour or more in each fluid. Embryos in formalin must be rinsed in water for one hour.

2. Stain in dilute **borax carmine** one day or more.

Borax, 4 per cent aqueous solution	100 cc.
Carmine	1 gr.
Boil until dissolved and add Alcohol, 70 per cent	100 cc.

To dilute, take 5 cc. of the borax carmine and 95 cc. of 35 per cent alcohol and add a crystal of thymol.

3. If overstained, remove the surplus color with hydrochloric acid (1 per cent solution in 70 per cent alcohol) after passing through water and 50 per cent alcohol, an hour each.

4. Run up through 80, 95 and 100 per cent alcohol, an hour each, and place in xylol until transparent.

5. Prepare a mounting diagram by drawing an outline of a slide on a piece of cardboard and in this laying off an outline of the cover glass to be used. Place a clean slide on the diagram, and, just inside the right and left margins of the cover-glass outline, attach a thin strip of celluloid, 15/1000 of an inch in thickness, by means of a drop of acetone. Greater thicknesses may be obtained by attaching other strips as necessary. When these supports are dry, place a few drops of Canada balsam, dissolved in xylol, between the supports, place the embryo in position, and lower a clean cover glass gently. Try to avoid the formation of air bubbles. If these appear later they may be removed by a needle which has been heated or dipped in xylol. A little fresh balsam may be run into the cavity.

The chick. — Total mounts may be stained either with the borax carmine or with Conklin's modification of **Delafield's**

hæmatoxylin. This stain, which gives a blue color to the embryo, is made as follows:

Hæmatoxylin (16 per cent solution in 100 per cent alcohol)	25 cc.
Ammonia alum (saturated aqueous solution)	400 cc.
Hydrogen peroxide, neutralized	25 cc.
Glycerin	100 cc.
Alcohol methyl	100 cc.

Conklin's modification consists of diluting the stain with four times the volume of distilled water and adding to each 100 cc. of the dilute stain 1 cc. of picrosulphuric acid, prepared by adding to 98 cc. of picric acid (saturated aqueous solution), 2 cc. of sulphuric acid.

1. Run the embryo from the 80 per cent alcohol down to water through changes of 70 and 50 per cent alcohol, an hour each.

2. Stain in borax carmine, undiluted, over night, or in hæmatoxylin from one to three hours. Either stain may be diluted still further and the staining period prolonged. In the author's laboratory the schedule demands a four-day staining period and the borax carmine is diluted 5×, the hæmatoxylin 20×.

3. Destain, if necessary, in acid alcohol until the desired color is obtained. Embryos stained with hæmatoxylin will turn red in the acid alcohol, and the blue color must be restored by washing them in running water or, after washing in neutral 70 per cent alcohol, placing them in alkaline alcohol (1 per cent ammonia in 80 per cent alcohol).

4. Run up the alcohols, 80, 95 and 100 per cent, half an hour each. Pour off half the 100 per cent alcohol and add an equal amount of xylol. When the diffusion currents disappear, transfer to pure xylol and leave until the embryo is transparent. In rainy weather, or when 100 per cent alcohol cannot be obtained, carbol-xylol (carbolic acid crystals, 25 gr. and xylol 75 cc.) may be substituted.

5. Remove the embryo from the cover glass (if it has not already detached itself) and trim the surrounding blastoderm to the form of an oblong or circle. Arrange a clean slide on the mounting diagram, as described for the frog, attach celluloid support, and mount the embryo in Canada balsam with the same side uppermost that was in that position when the egg was

opened. Put the slide away where it may lie flat and free from dust until the balsam has hardened. This will take at least a week, after which the slide may be cautiously cleaned and studied. The process may be hastened by drying the slide in the paraffin oven.

The pig. — Embryos up to 10 mm. body length may be prepared as whole mounts by staining in dilute borax carmine, destaining until only a trace of color persists, and mounting in Canada balsam. The time spent in each alcohol should be at least an hour for the larger embryos.

D. SERIAL SECTIONS

In the preparation of serial sections of an embryo, the fixed material is (1) **embedded** in a suitable matrix and (2) sliced into extremely thin **sections,** which are (3) **mounted** in serial order upon slides. The embryo may be stained before or after sectioning.

Embedding. — There are two principal methods of embedding, in paraffin or in celloidin. For especially delicate objects, the best results are obtained by a combination of these methods, the embryo being first impregnated with celloidin in order to avoid the shrinkage (about 10 per cent) caused by paraffin embedding, and the block of celloidin then immersed in paraffin so that ribbons of serial sections may be cut.

Embedding in paraffin. — In preparing the first few embryos for sectioning, it is advisable to stain, dehydrate, dealcoholize, and clear as if for a total mount. Later, the staining may be omitted until after the sections are affixed to the slide.

1. After clearing in xylol, which should be done in a warm place, for example, the low-temperature oven at about 40° C., pour off half the xylol and add an equal amount of paraffin chips. In the author's laboratory a paraffin of about 55° melting point, obtained by mixing commercial paraffin with parawax, is used. The parawax, unfortunately, varies in melting point, so that the formula is empirical. The embryo may be left in this xylol paraffin for two days.

2. If the mixture has hardened it should again be melted in the low-temperature oven. Fill a clean Stender dish with melted paraffin, transfer the embryo to this, and place in the high-tem-

perature oven at about 56° C. (or one degree above the melting point of the paraffin used) for not more than two hours. The xylol paraffin should be thrown in the slop jar. Take care not to get any xylol in the high-temperature oven or paraffin used for the final embedding.

3. Smear the interior of a small watch glass with a 10 per cent aqueous solution of glycerin, and fill with fresh melted paraffin. Transfer the embryo to this, making any necessary adjustments in position with a heated needle. Place the embryo dorsal side up, and note the position of the head. Cool the surface of the paraffin by blowing on it gently until it is congealed. Then plunge it immediately into a dish of cold water or waste alcohol and leave it there for five minutes. Mark the block for identification. Objects may be left in paraffin indefinitely.

4. On removing the block of paraffin from its container, examine for the following flaws:

a. Air bubbles, if they are not near the embryo, may be removed with a hot needle. Otherwise it is better to trim the block close to the embryo, put it into melted paraffin, and re-embed.

b. Milky streaks are due to the presence of xylol. These will crumble during sectioning, so that it is best to re-embed if they occur near the embryo.

c. If the paraffin has " fallen " in the center, it is because the surface was cooled too long before the block was immersed in the water. If any part of the embryo is exposed, it must be re-embedded.

Sectioning after paraffin embedding. — Before sectioning your first embryo, be sure you understand the mechanism of the microtome (there are many varieties, of which the rotary type is best adapted to beginning students), and have practised the technique on a block of paraffin. There are three standard planes of sectioning corresponding to the axes of the body (Fig. 194). **Transverse** sections are obtained by cutting the cephalic end of the body first, with the knife entering the left side. **Sagittal** sections are made by cutting the right side first, with the knife entering the ventral surface. **Frontal** sections are made by commencing at the ventral surface, the knife entering the left side. It is best to begin with transverse sections.

1. Attach the paraffin block to the object-carrier of the microtome in the proper manner to obtain the type of section desired. This is done by heating the surface of the carrier until it will just melt paraffin, pressing the block against it in the desired orientation, and lowering into a dish of cold water. A little melted paraffin may be poured around the base of the block and this again cooled to secure additional support.

2. Place the object-carrier in the microtome and, after orienting the block with respect to the knife, trim it so that the end of the block is a perfect rectangle with one of the longer sides parallel to the knife edge. If one of the angles is cut off slightly

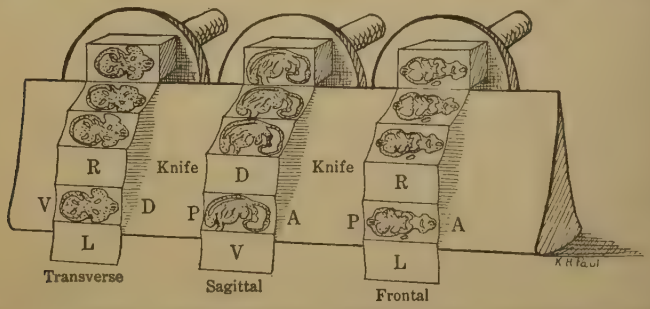

FIG. 194. — Diagram showing method of orienting embryo on microtome to secure transverse, sagittal and frontal sections.

there will be a series of indentations in the ribbon which will assist in orienting the sections on the slide.

3. If microtome knives are not available, place a new safety-razor blade (Autostrop type) in the holder provided, allowing the edge to project between a sixteenth and an eighth of an inch. Screw the holder in the knife-carrier so that the edge of the blade is tilted inward about 10° from the perpendicular.

4. Set the regulator for 20 microns (thousandths of a millimeter).

5. Run the feed screw as far back as it runs freely; do not force it.

6. Advance the knife-carrier until the edge of the blade just clears the block.

7. Release safety catch and turn the wheel steadily until the knife begins to cut the block. Cut slowly, making necessary

adjustments to the block and knife until you are cutting a perfectly straight ribbon without wrinkles or splits. The principal causes of trouble and their remedies are as follows:

a. The ribbon curls to right or left. This happens because (1) the block is thicker on the side away from which the ribbon curls, or (2) the knife is duller on the side toward which the ribbon curls. Remedy: (1) trim the sides of the block parallel; (2) shift the knife to one side.

b. The sections curl and the ribbon is not continuous. This is due to (1) too much tilt of the knife, (2) too hard a grade of paraffin, or (3) too cold a room. Remedy: (1) lessen tilt of knife; (2) re-embed in softer paraffin; (3) move microtome to warmer place, light an electric light, or micro-bunsen burner near microtome, or cut thinner sections.

c. The ribbon wrinkles badly. This is caused by (1) too little tilt to the knife, (2) too soft a grade of paraffin, (3) too warm a room, or (4) a dull or dirty knife. Remedy: (1) increase the tilt of the knife; (2) re-embed in harder paraffin; (3) move to a cooler room, or cool the knife and block by dropping alcohol on them and blowing vigorously, or cut thicker sections; (4) clean knife edge with cloth moistened in xylol or shift to a new place on the knife.

d. The ribbon splits lengthwise. This is due to (1) a nick in the knife, (2) a bubble in the paraffin, or (3) dirt on the knife edge or side of the block. Remedy: (1) shift to new cutting edge; (2) paint surface with thin celloidin; (3) clean knife edge and block.

e. The sections refuse to ribbon; they fly apart or cling to the knife or the block. This is due to the electrification of the sections caused by unfavorable atmospheric conditions. Many remedies have been suggested; the best is to ground the microtome to a water pipe. Usually it is advisable to wait for more favorable conditions.

8. Remove the ribbon in 6-inch lengths with a camel's hair brush and arrange these in order, shiny side down, in a cardboard box cover. Avoid air currents of all kinds. The ribbons may be put away in a dust-free place if the room is not too warm. It is better to affix them to slides as soon as possible.

Affixing paraffin sections to the slide. — 1. Prepare a **mounting diagram** by laying off the outline of a slide as before, but enclose

in this the outline of a long cover glass (25 × 50 mm. approximately) and leave room for a label on the right-hand side.

2. Clean a slide thoroughly by washing with acid alcohol followed by distilled water. Place this over the mounting diagram and brush over the surface above the outline of the cover glass with the following dilute solution of **egg albumen:**

Egg albumen, beaten and skimmed	50 cc.
Glycerin	50 cc.
Filter and add Thymol	a crystal
Dilute 3 drops of this to distilled water	10 cc.

3. Cut the ribbon into lengths about 2 per cent shorter than the length of the cover glass. Using the wet brush from which most of the albumen solution has been squeezed, pick up these lengths and arrange them on the albumenized slide so that the sections will follow each other like the words on a printed page. The shiny side of the ribbon should be next to the slide. Great care should be taken to prevent the formation of air bubbles beneath the ribbon.

4. Carefully warm the slides on a warming plate or a piece of plate glass, previously heated in the paraffin oven, until the sections are expanded and perfectly smooth. If bubbles appear beneath the ribbon, prick them with a needle. Remove the surplus water with bibulous paper, mark the slides with a glass-marking crayon, and set them away in the low temperature oven to dry, at least two days. They may be kept indefinitely in this condition if not exposed to dust.

Embedding in celloidin. — This method is preferred by some technicians as no heat is used in the process and the shrinkage is less than that resulting from the paraffin method. However, thin sections are not so easy to obtain and the sections must be handled individually.

1. Embryos are dehydrated as for the paraffin method. Leave in absolute alcohol one day.
2. Absolute alcohol and ether, equal parts, one day.
3. Thin celloidin, three days to one week.

Alcohol, 100 per cent	100 cc.
Ether	100 cc.
Celloidin	5 gr.

4. Thick celloidin, two days to two weeks.

Alcohol, 100 per cent	100 cc.
Ether	100 cc.
Celloidin	15 gr.

5. Remove the embryo to a small watch glass and pour thick celloidin over it. Cover lightly, or place under a bell jar until the celloidin is hard enough to cut with a scalpel.

6. Dip a block of vulcanized fiber in thick celloidin. Cut out a block of celloidin containing the embryo from the watch glass and, after moistening the end by which it is to be attached in ether alcohol, press it firmly against the prepared fiber block.

7. Pour a little chloroform into a Stender dish, add the block and embryo, cover tightly, and allow the celloidin to harden in the fumes for thirty minutes.

8. Fill the Stender dish with chloroform and cover. Leave for thirty minutes.

9. Pour off half the chloroform and add an equal amount of cedar oil. Leave for one hour.

10. Transfer to pure cedar oil where it may remain indefinitely.

Sectioning after celloidin embedding. — Celloidin sections are usually cut with some form of sliding microtome. Be sure to study the mechanism and cut a piece of hardened celloidin before proceeding further.

1. Set the knife with a little more tilt than would be used for paraffin, and obliquely to the object so that at least half the cutting edge will be drawn through the block.

2. Orient the block upon the object-holder so that the desired type of sections may be obtained. The long side of the block should be parallel to the edge of the knife.

3. Cut sections 20 μ or more in thickness, using a steady drawing cut. Mount sections as they are cut.

Affixing celloidin sections to the slide. — This is best done as the sections are cut.

1. Using the mounting diagram as before, rub on a thin film of undiluted albumen solution to cover the areas of the cover glass. Rub in well with the ball of the finger.

2. Arrange the sections in order on this area. When this is filled, lay a cigarette paper over the sections and press gently

with another slide. The slides may be kept in a dust-free container.

Double embedding in celloidin and paraffin. — This process, although tedious, combines the best points of the two methods already given.

1. Embed in celloidin according to the method above, omitting step 6.
2. Trim the celloidin block close to the embryo and wash out the cedar oil with xylol, three changes in two hours.
3. Embed in paraffin as described above, commencing at step 2.
4. Section according to the method given for paraffin.
5. Affix to the slide according to the method given for paraffin sections.

Staining serial sections. — When the embryo has been stained before sectioning, it is only necessary to remove the paraffin (or celloidin), replace with Canada balsam, and cover, if the stain proves to be satisfactory. Sometimes, however, it is advisable to strengthen or weaken the stain or to add a contrasting dye.

After staining in bulk. —

1. **Paraffin sections** on the slide should be put in a Coplin staining jar of xylol and left until the paraffin is dissolved. Up to fifteen minutes.
2. Transfer to a mixture of xylol and 100 per cent alcohol, equal parts, five minutes.
3. Transfer to 100 per cent alcohol, five minutes.
4. Examine slide rapidly under microscope after wiping the back of the slide.

a. If the stain is satisfactory,

5*a*. Absolute alcohol-xylol, five minutes.
6*a*. Xylol, ten minutes.
7*a*. Mount in balsam under cover glass.

b. If the stain is too intense,

5*b*. Ninety-five and 85 per cent alcohol, one minute each.
6*b*. Acid 70 per cent alcohol, until stain is correct.
7*b*. Sections stained in hæmatoxylin should have the blue color restored in alkaline 85 per cent alcohol.
8*b*. Eighty-five, 95 and 100 per cent, one minute each.
9*b*. Absolute-xylol, five minutes.

10*b*. Xylol, ten minutes.
11*b*. Mount in balsam.

c. If the stain is too light,

5*c*. Ninety-five, 85, 70 and 50 per cent alcohol, one minute each.
6*c*. Stain until desired effect is secured.
7*c*. Distilled water, five minutes.
8*c*. Fifty, 70, 85, 95, 100 per cent alcohol, one minute each.
9*c*. Absolute-xylol, five minutes.
10*c*. Xylol, ten minutes.
11*c*. Mount in balsam.

Celloidin sections on the slide should be exposed to the fumes of the alcohol-ether mixture for half a minute, dried for one minute, and placed in a Coplin jar of 95 per cent alcohol. All other operations may be carried on as above except that carbol-xylol should be substituted for 100 per cent alcohol.

Counterstaining after staining in bulk. — In order to differentiate the parts of the embryo more sharply, it is often desirable to add a second stain contrasting with the first. The stains that have been employed in the previous exercises are nuclear dyes; that is, when extracting by acid alcohol, the color will persist in the nucleus after it has been washed out of the cytoplasm. The second stains affect the cytoplasm and should contrast in color with the nuclear stain employed. After borax carmine, a 0.5 per cent solution of anilin (Lyons) blue in 95 per cent alcohol is employed; after hæmatoxylin, a similar solution of eosin should be used.

1. Proceed as in the preceding section as far as 6*b*.
2. Destain in acid alcohol until the color persists only in the nuclei.
3. Restore the blue color to hæmatoxylin-stained sections in alkaline 80 per cent alcohol.
4. Eighty and 95 per cent alcohol, one minute each.
5. Counterstain lightly, dipping the slide into the solution repeatedly until a light color persists in the sections, one-half to one minute.
6. Rinse in 95 per cent alcohol, dehydrate with 100 per cent alcohol, followed by xylol-absolute, clear in xylol and mount.

Staining with Delafield and eosin on the slide. — Follow directions given for sections stained in bulk (where stain is too light), as far as step 6c, and follow with directions for counterstaining as given above.

Staining with Heidenhain's hæmatoxylin. — This is one of the most important embryological stains.

1. Remove the paraffin from the sections and run down the alcohols to distilled water.
2. Four per cent aqueous solution of iron alum, one hour to over night.
3. Rinse in distilled water and place in 0.5 per cent aqueous solution of hæmatoxylin, same time as in the iron alum.
4. Rinse in distilled water and return to the iron alum until sections are a pale grey. Check from time to time by rinsing in distilled water and examining under microscope to see that the desired structures are still visible.
5. When sufficiently destained, wash in running water for twenty minutes, or in distilled water, with frequent changes, for two hours.
6. Run up the alcohols, clear, and mount.

Fuchsin and picro-indigo-carmine. — This polychromatic stain is especially fine for organogeny.

1. Remove the paraffin and run down the alcohols to distilled water.
2. Stain in basic fuchsin, saturated aqueous solution, twenty minutes.
3. Rinse in distilled water and place in picro-indigo-carmine for five minutes.

Picric acid, saturated aqueous solution	50 cc.
Indigo-carmine saturated aqueous solution	50 cc.

4. Pass rapidly through 70, 95 and 100 per cent alcohol into xylol-alcohol. The green dye is extracted most rapidly by the 70 per cent alcohol, the red by the 100 per cent. Only experience will teach the right time allowance for each alcohol.
5. Clear in xylol, and mount.

Oppel's polychromatic stain. — This gives beautiful effects with older embryos and larvæ.

1. Fix in Bouin.

2. Stain in bulk with undiluted borax-carmine, one to two days. Destain for the same period.
3. Embed, preferably by the double method.
4. Cut sections, 15–20 microns.
5. Run down the alcohols to water.
6. Stain in picro-indigo-carmine, 1½ minutes.
7. Stain in picro-fuchsin, one minute.

Picric acid, saturated aqueous solution	50 cc.
Acid fuchsin, saturated aqueous solution	50 cc.

8. Wash in distilled water, changed repeatedly, five minutes.
9. Ninety-five per cent alcohol, two minutes.
10. Carbol-xylol, xylol and mount.

E. TECHNICAL RECORDS

Not the least important part of technique is the keeping of exact records covering every technical operation. For each embryo there should be a card, giving the following data:

1. Kind of embryo and stage of development.
2. Method of fixation, time and date.
3. Bulk staining, time and date.
4. Method of embedding, time and date.
5. Type and thickness of sections, and date.
6. Slide staining, time and date.
7. Method of mounting, and date.
8. Name of preparator.

F. OUTLINE OF TECHNICAL PROCEDURE FOR CHICK EMBRYOS

1. Remove embryo from egg in warm normal salt solution.
2. Fix for two hours in Bouin's fluid.
3. Wash in 70 per cent alcohol (plus lithium carbonate), at least one change, for two days.
4. Pass through 50 per cent alcohol and water, one hour each.
5. Stain in dilute borax-carmine or Delafield's alum-hæmatoxylin, four days.
6. Destain in acid 70 per cent alcohol until desired effect is obtained.
7. Wash in neutral 85 per cent alcohol. (The hæmatoxylin-

stained specimen is transferred to alkaline 85 per cent alcohol until blue color is restored.) Two days.

8. Dehydrate and clear: 95 per cent, 100 per cent alcohol, absolute-xylol mixture, xylol, twenty minutes each.

Mount in Canada balsam

OR

9. Prepare for embedding by pouring off half the xylol and adding an equal amount of paraffin chips. Keep in warm place up to four days.

10. Continue by transferring embryo to melted paraffin and place in paraffin oven for an hour and a half.

11. Embed in fresh paraffin and cool in water. Make blocks.

12. Cut transverse sections 20 μ in thickness on microtome.

13. Prepare clean albumenized slide, float sections on this in order, warm until sections are expanded, remove surplus water. Dry for at least two days.

14. Remove paraffin with xylol, and

A. Mount in balsam, or

B. Run down alcohols to 70 per cent and destain. Run up the alcohols, through absolute-xylol and xylol, mount in balsam, or

C. Run down alcohols to water and restain, dehydrate, clear and mount, or

D. To 95 per cent and counterstain for one minute. Dehydrate, clear, and mount.

REFERENCES

Ballentyne, F. M. 1928. An Introduction to the Technique of Section Cutting.
Gage, S. H. 1925. The Microscope, 14th Ed.
Guyer, M. F. 1917. Animal Micrology, 2nd Ed.
Lee, A. B. 1929. The Microtomist's Vade-Mecum, 9th Ed.
McClung, Ch. 1929. Handbook of Microscopical Technique.
Oppel, A. 1914. Embryologisches Practikum und Entwicklungslehre.
Shumway, W. 1926. Fuchsin and Picro-indigo-carmine, a Polychromatic Stain for Vertebrate Organogeny. Stain Technology I, 1.

CHAPTER XII

STUDY OF EMBRYOLOGICAL PREPARATIONS

During the early stages of development, embryos are too small to be studied with the unaided eye. Some observations, to be sure, may be made with the dissecting lens, but most embryological work requires the use of the compound microscope. Although the student may be familiar with the use of the microscope from the elementary course in biology, he should nevertheless review this subject before proceeding further. In addition, he should at this time familiarize himself with the simpler methods of measuring objects with the aid of the microscope, as embryological drawings require a strict accuracy as to proportions. A great convenience in embryological work is the camera lucida or some other device by means of which accurate outlines may be traced. Finally, we must consider the methods by which the embryo may be reconstructed in magnified form from serial sections, thus returning, in a sense, to the point where the study of embryological technique was begun.

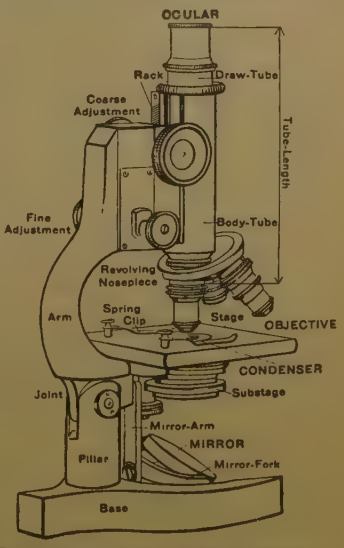

FIG. 195. — Diagram of the microscope. (From Gage.)

A. THE USE OF THE MICROSCOPE

Nomenclature of the microscope. — The separate parts of the microscope (Fig. 195) may be grouped into two systems, the mechanical parts, and the optical parts. The principal mechanical parts are the base, from which arises the pillar, attached to which is the arm, which may be inclined at the joint. Attached to the arm, just above the joint, is the stage, upon which the

slide is placed for examination, and beneath this, the movable sub-stage equipment, consisting of a condenser-sleeve, and one or two iris-diaphragms, by means of which the amount of light to be used is regulated. At the base of the arm is the mirror, a silvered double mirror, with a plane surface on one side and a concave surface on the other. At the upper end of the arm are two screws, the coarse and fine adjustments, by means of which the barrel of the microscope may be raised or lowered either rapidly or very slowly. The barrel is composed of the body-tube, connected to the arm by a rack and pinion, in the upper end of which is enclosed an inner tube, the draw-tube, on which is a graduated scale of millimeters representing the tube length exclusive of the revolving nose-piece at the lower end. The optical parts of the microscope are systems of lenses, the condenser, placed in the condenser-sleeve, the objectives, attached to the revolving nose-piece, and the oculars, one of which is placed at the upper end of the draw-tube.

The condenser. — This is a system of lenses which increases the amount of illumination thrown upon the object, and is required only with the higher-power objectives.

The objectives. — These are systems of lenses which produce an enlarged and inverted image of the object under proper conditions. Objectives were formerly marked by arbitrary letters or numbers, with the lowest-power objectives beginning the series. To-day they are usually indicated by the equivalent focal length (E. F.), that is, the focal length of a simple lens at 250 mm. or 10 inches, or else by the actual magnification (×) at 160 mm., (Leitz microscopes, 170 mm.). The most useful objectives for general embryological purposes are the 25-mm. or 6×, which will hereafter be spoken of as the lower-power objective; the 16-mm. or 10×, which will be called the medium-power objective; and the 4-mm. or 40×, known as the high-power objective. For the study of the germ cells, an oil-immersion objective, of which the front lens must be in contact with the cover glass by means of a drop of cedar oil, is necessary. The most generally used immersion objective is that of 1.9 mm. E. F. or approximately 95×.

Oculars. — These are systems of lenses which magnify the real image formed by the objective. Like objectives, these

were, in the past, usually numbered or lettered beginning with that of the lowest power, but now are marked with the E. F. at 250 mm. or the actual magnification at 160 mm. (Leitz oculars, 170 mm.). The most useful oculars are the 50-mm. (5×) or low-power ocular, and the 25-mm. (10×) or high-power ocular. When used with the objectives given above, a range of magnification of from 30× to 450× may be obtained. A method of obtaining the exact magnification will be described in connection with the directions for reconstruction given below.

The use of the microscope. —

1. Place the microscope squarely in front of you with the pillar toward you and the stage horizontal.

2. Place the low-power ocular in the draw-tube and adjust this to a length of 160 mm. (170 mm. for Leitz instruments) as indicated on the millimeter scale. Swing the low-power objective into position. Place the mirror bar in the median line and adjust the mirror to secure an even illumination. Use the plane side of the mirror for daylight, the concave side for artificial light.

3. Place the slide on the stage so that the object to be examined is in the center of the stage aperture, and fasten it down with the spring clips provided. With the coarse adjustment, lower the body-tube until the objective nearly touches the cover glass. Then, with the eye at the ocular, slowly raise the body-tube until the object comes into plain view. With the fine adjustment, raise and lower the body-tube a little at a time until the point at which the smallest details show clearly is discovered. This is the focal point.

4. When using the low-power and medium-power objectives, the condenser should be lowered until the illumination is evenly distributed. With the high-power objective, the condenser should be raised almost to the level of the stage. The iris-diaphragm should be open sufficiently to illuminate about three-quarters of the aperture of the objective. In other words, it is more widely open for the low-power objective than for the high-power objective.

5. If a greater magnification is desired, change to the high-power ocular, which will double the magnification. If this is not sufficient, return to the low-power ocular and swing the medium-

power objective into position, and so on. On most modern instruments, the objectives are par-focal; that is to say, the lengths of the objectives are such that when another objective is swung into place the object will still be visible. If, however, the object is not in focus, it is best to lower the body-tube until the new objective almost touches the cover glass, and focus up until the object comes into view. If the oil-immersion objective is to be used, lower the condenser and place a drop of oil on its upper surface; then raise it until it touches the bottom of the slide. Place another drop immediately over the object on the cover glass and lower the body-tube with great care until the front lens of the objective touches the oil. Focus by means of the fine adjustment only.

6. All optical parts of the microscope must be cleaned with lens-paper. After the oil-immersion objective has been used, the front lens, condenser, and slide should be wiped with a bit of lens-paper dipped in xylol and then dried with a fresh piece. Never separate any of the optical parts. The microscope should be lifted by the pillar unless a special grip is provided to the arm. The microscope should be kept in the case when not in use. One of the oculars should be left in the draw-tube at all times to prevent dust getting on the upper lenses of the objectives. Beginners should try to avoid the error of closing the eye that is not in use. Practice will enable the microscopist to work with both eyes open and even to alternate the right and left eye at the ocular.

Micrometry. — The unit of measurement in microscopy is the micron (μ). It is the one-thousandth part of a millimeter. Measurement of microscopic objects is performed with the aid of micrometers, of which there are two types, the stage micrometer and the ocular micrometer. The former is a glass slide, in the center of which, under a cover glass, is a line, usually 2 mm. long, divided into 200 equal parts, each of which, therefore, is equivalent to 10 μ. The ocular micrometer is a glass disc, placed in an ocular at the level of the ocular diaphragm, on which is engraved a scale, with arbitrary subdivisions. Some oculars are furnished with a draw-tube so that the upper lens of the system may be focused more sharply upon the scale. The value of the divisions indicated on the scale varies according to the amount of magnifi-

cation of the real image, and so must be obtained for each objective independently, according to the following method:

1. Arrange the microscope as before, taking particular care to secure the proper tube-length.

2. Focus the eye-lens on the ocular micrometer scale by means of the ocular draw-tube. Focus the objective on the stage micrometer.

3. Make the lines of the stage micrometer parallel with those of the ocular micrometer, and determine the value of the divisions of the ocular micrometer in terms of those of the stage micrometer. Thus, if it requires 10 spaces of the ocular micrometer, and the latter is equal to 0.1 mm., then the value of a single space of the ocular micrometer for that particular objective and at that particular tube-length is 0.01 mm. or 10 μ. Determine the value of the ocular micrometer for each objective in the same way.

B. EMBRYOLOGICAL DRAWINGS

Free-hand drawings of microscopic objects can only approximate an accurate representation. However, great pains should be taken to secure at least accurate proportions, neat and clean-cut lines, and complete labels. Accurate outlines can be secured by the aid of the camera lucida, various types of projection apparatus, or microphotography.

Equipment. — The student will need a hard lead pencil (4H), a medium pencil (HB), and blue, red, and yellow colored pencils, an eraser, and bond paper to fit the note-book cover used in earlier courses.

Free-hand drawing. —

1. Lay off the space to be occupied by the drawing, by placing four dots at the corners. Rule in two lines, intersecting at the center of this space. These will represent the dorso-ventral and the dextro-sinistral axes, if the drawing is to be of a transverse section.

2. Measure the corresponding axes of the sections by means of the ocular micrometer, multiply by the desired magnification of the drawing, and lay off these magnified measurements on the cross lines already drawn. The following magnifications are recommended: for the twenty-four-hour chick, 100×; for the

thirty-three-hour chick, 75×; for the forty-eight-hour chick, 50×; for the seventy-two hour chick, 30×; for the 10 mm. pig, 20×.

3. Draw in a careful outline of the section and of the internal structures, paying particular attention to the proportions, which should be measured with the ocular micrometer and laid off on the axes at the proper magnification.

4. On one side of the dorso-ventral axis, all structures should be colored with the crayons in accordance with the following scheme: ectoderm, blue; mesoderm, red; and endoderm, yellow.

5. Label all structures represented in the section, using broken lines at right angles to the long axis of the paper to connect the label with the structure indicated.

6. Identify the drawing fully, by means of a serial number, the species, and stage of development, the number given to the series, slide and section, the type of sections, and the amount of magnification. Example: No. 23, Chick, 48 hours, Series 1102, Slide 2, section 28, transverse section 50×. If a drawing has already been made of the total embryo or a total mount, indicate on this, by means of a heavy ruled line, the position of the section just drawn, and number this line with the serial number of the section.

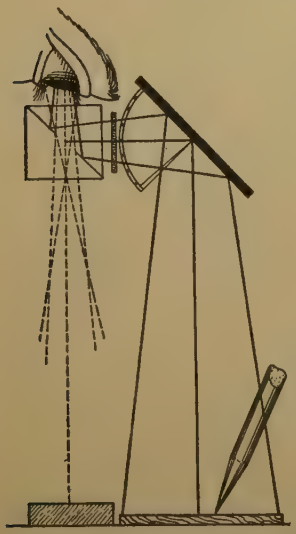

Fig. 196. — Diagram to illustrate method of drawing with camera lucida. (After Gage.)

Abbé camera lucida. — This is an attachment which reflects the light from the drawing board, by means of a mirror, to a silvered prism, whence the light is reflected to the eye, superimposed on the image of the object which is transmitted through a small hole in the silvered surface of the prism directly above the ocular of the microscope (Fig. 196).

1. Attach the camera to the draw-tube of the microscope in such a way that the mirror projects to the right, and the opening in the prism lies above the center of the ocular.

2. Extend the mirror arm to its greatest length and set the mirror at an angle of 45°. The mirror arm must be parallel to the drawing board.

3. Try various combinations of objectives and oculars until an image of the desired magnification appears on the paper. Magnifications intermediate to those obtainable in this way may be secured by varying the tube-length or by raising or lowering the drawing board. If the stage of the microscope interferes with the drawing, the mirror should be set at an angle of 40° or 35° and the drawing board tilted toward the microscope at an angle of 10° or 20°, respectively, by means of wooden images. If the image is stronger than the reflection of the pencil point, a smoked glass may be placed beneath the prism or the aperture of the iris diaphragm may be reduced. If the reflection of the pencil is stronger than the image, smoked glass may be placed at the side of the prism or the amount of light falling on the paper reduced by means of a screen.

4. Draw in the outlines of the sections and the larger internal structures. The details may be added free-hand.

5. Remove the slide and substitute a stage micrometer. Trace in part of the scale by means of which both the magnification of the drawing and the absolute size of the object may be computed readily.

Projection apparatus. — Where many drawings are to be made, as in the case of reconstructions, some form of apparatus by means of which the image of the section may be projected directly upon the paper is very helpful. There are many types of projection apparatus, directions for the use of which may be obtained with the instruments.

Microphotography. — The photography of minute objects with the aid of the microscope is of great assistance in embryology. However, the methods are so difficult, the apparatus so complex, expensive, and delicate, and the process requires so much technical knowledge and skill, that microphotography has been considered a field too advanced for the beginning student. A method recently described by Headland seems to overcome these difficulties to a large extent.

C. RECONSTRUCTION

After an embryo has been sectioned, it is sometimes necessary to reconstruct some part of it from the sections. There are two important methods: graphic reconstruction, in which a geometric projection of a sagittal section, for example, might be made from transverse sections; and plastic reconstruction, in which magnified replicas of each section are made of wax and piled together so as to make an enlarged model of the object to be studied. A complete series of sections of uniform thickness and accurate orientation is required for either type of reconstruction, and an outline drawing of the embryo before sectioning is of great assistance.

The graphic method (of His). — This method can best be described by giving practical directions for a particular problem, e.g., to prepare a geometrical sagittal projection 20× of the neural tube of a 10 mm. pig embryo from a series of transverse sections 20 μ in thickness.

1. From the lateral view of the embryo drawn before sectioning, make an outline drawing 20×.
2. Draw a median line corresponding to the cephalo-caudal axis, the length of which, in this case, should be 200 mm.
3. Count the number of sections in the series, in this case, 500.
4. Locate the position of each transverse section which you have drawn on the median line of the outline. Thus if the most anterior section drawn was the fiftieth of the series of 500 sections, it would be located at a point 1/10 of the total length of the axis (200 μ), or 20 mm. from the cephalic end.
5. Theoretically, each section is at right angles to the median line, but this angle may be greater or less as a result of variations in technique. Study each drawing of a cross section in connection with the drawing of the total embryo and determine the angle made by that section with the cephalo-caudal axis of the embryo. Draw in, at each point located on the median line, a cross line at the proper angle so determined. These lines represent the dorso-ventral axes of the transverse sections. Their lengths should correspond with those of the dorso-ventral axes of the drawings of the transverse sections previously made at the same magnification, 20×.

6. Plot in on each section-plane line (dorso-ventral axis) the dorsal and ventral boundaries of the neural tube as determined from measurements of the drawings already made. Interpolate by direct measurement and magnification of these points on intervening sections.

7. Sketch in the contours of the neural tube by connecting up the points which have just been plotted. Compare the drawing with a sagittal section of an embryo in the same stage of development.

Plastic reconstruction. — This method also will be indicated by practical directions for the reconstruction of a particular organ, in this case, a model 50× of the heart of a 10 mm. pig, from a series of transverse section, 20 μ in thickness.

1. Prepare a number of wax plates of the proper thickness. In this case, if every section is to be reconstructed, the thickness of the plates must be 50× 20 μ, or 1 mm. Nearly as good results can be obtained by reconstructing every second section and making the plates twice as thick. The wax is prepared according to the following formula:

Beeswax	6 parts
Paraffin, 56° C. m.p.	4 parts
Rosin, white lump	2 parts

Mix and melt.

Pour 130 grams of this wax into a pan with an inside measurement of 500 × 130 mm., into which boiling water has been poured to a depth of 15 mm. This amount of wax will make a plate 1 mm. in thickness. Bubbles in the wax may be removed by playing the flame of a bunsen burner over the surface as it is cooling. As the surface hardens, cut the edges free from the sides of the pan. When the wax has set but is still malleable, roll up the plate and remove it to a soapstone slab, where it is unrolled and allowed to cool.

2. With the help of a camera lucida or projection apparatus, prepare outlines 50× of the heart in all the sections in which it is found. Number each drawing consecutively and note the serial number of the sections drawn, so that it will be possible to check the drawings later if necessary. Note also whether the right and left sides of the drawing actually correspond with the right and left sides of the embryo or whether this condition is

reversed. This is very important, as a mistake at this point would render the reconstruction valueless.

3. Transfer the drawings to the wax plates by means of carbon paper. Place the wax plates on a sheet of glass and cut out the parts to be preserved with a sharp scalpel, leaving bridges of wax to connect the parts which would otherwise be separated. These bridges are best made in the form of arches bending towards the outside of the section.

4. Pile the sections in order, taking care to avoid the reversal of right and left sides, and to get an accurate fit. It is best to group the sections in piles of ten. A steady pressure of the hand will be sufficient to cause the sections to adhere to each other. The bridges may be cut away and stout pieces of wire substituted. Heat the wire at each end and press into position. After the wire is set, the wax bridges are cut away and the edges of the piece smoothed with a heated scalpel or aluminum modeling tool.

5. When all the sections have been combined in groups of ten, these groups should be united and the completed model smoothed in the same way. Such models may be painted or dissected, and mounted on wooden supports as desired. They are quite permanent if not exposed to high temperatures. Plaster of Paris molds and casts may be made from them in the customary manner.

REFERENCES

Gage, S. H. 1925. The Microscope, 14th Ed.

Guyer, M. F. 1917. Animal Micrology, 2nd Ed.

Headland, C. I. 1924. A Simple and Rapid Photomicrograph for Embryological Sections. Anatomical Record, XVII, 2.

Lee, A. B. 1929. The Microtomist's Vade-Mecum, 9th Ed.

Norman, J. R. 1923. Methods and Technique of Reconstruction. Journal of the Royal Microscopical Society.

INDEX

References to figures have an asterisk (*) prefixed to the page number. Many embryological terms not used in this text have been included in the index, accompanied by cross-references to the terminology employed by the author, e.g., Pharyngeal pouch, *see* Visceral pouch.

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